University of Essex

RNA-Seq II

Differential Gene Expression

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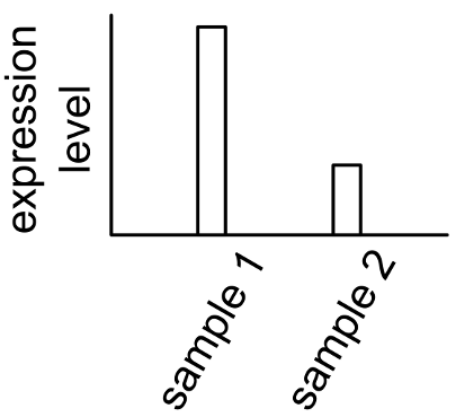
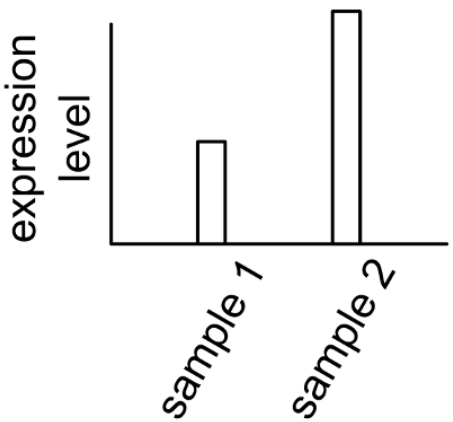
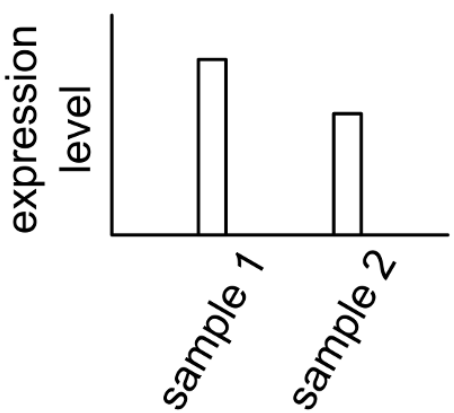
Outline

Comparison of two samples

Which of these pairs are significantly different?







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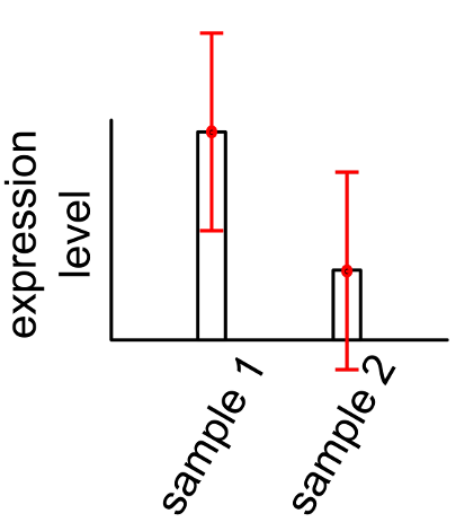

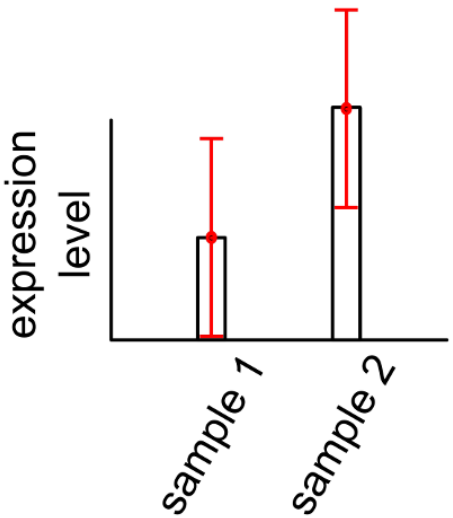

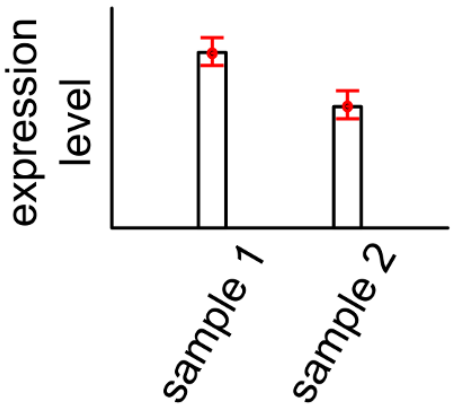

- Variability of a set of values is measured as the sample VARIANCE

$$V = \frac{1}{n-1} \sum_{i=1}^{n} (x_i - \bar{x})^2 \tag{1}$$

- If we know the variance we can estimate the Standard Error of the Mean (SEM)

Biological and technical replicates

To calculate Variances we need multiple measures of each gene:

- Biological replicates: variability among individuals
- Technical replicates: variability due to the method

Assessing differences

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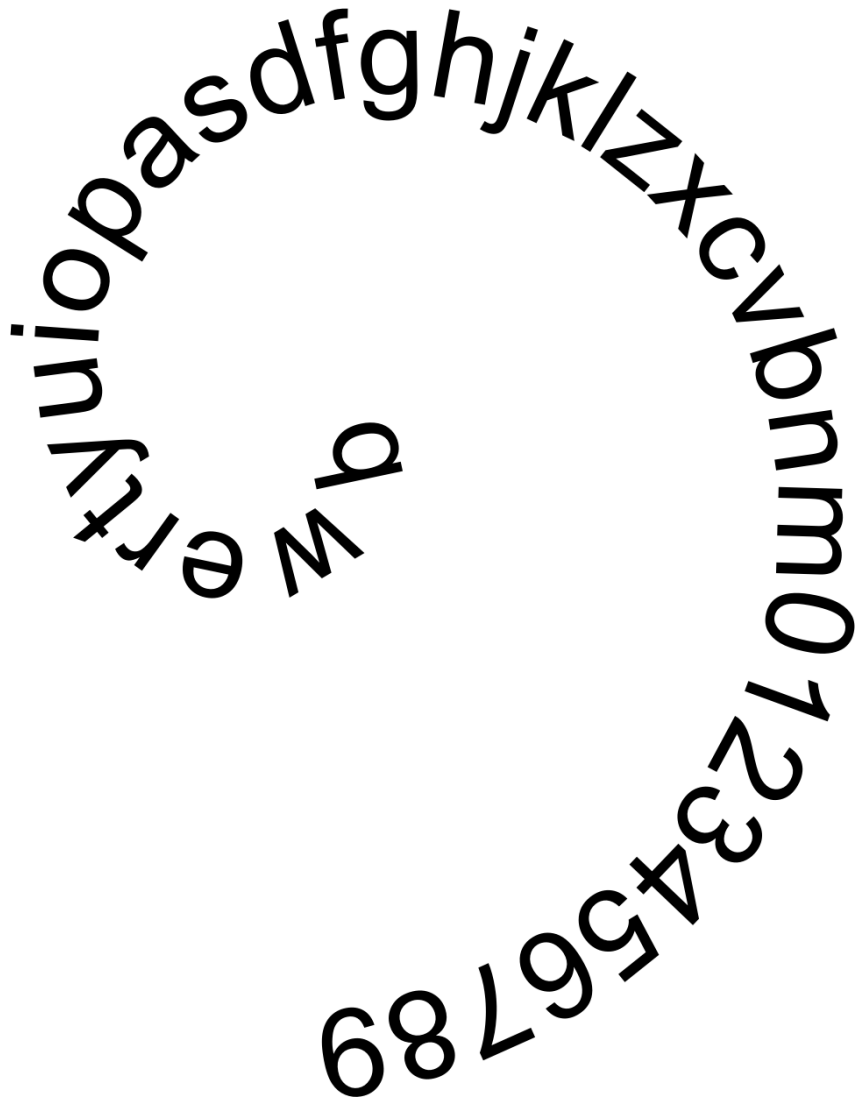
- Usually paired t-test, or a non-parametric test (Wilcoxon)
- Use ANOVA for more than 2 groups
- State-of-the-art methods use Generalized Linear Models
- For each gene we will have a p-value: the smaller it is, the more significant the difference

Outline

False positives



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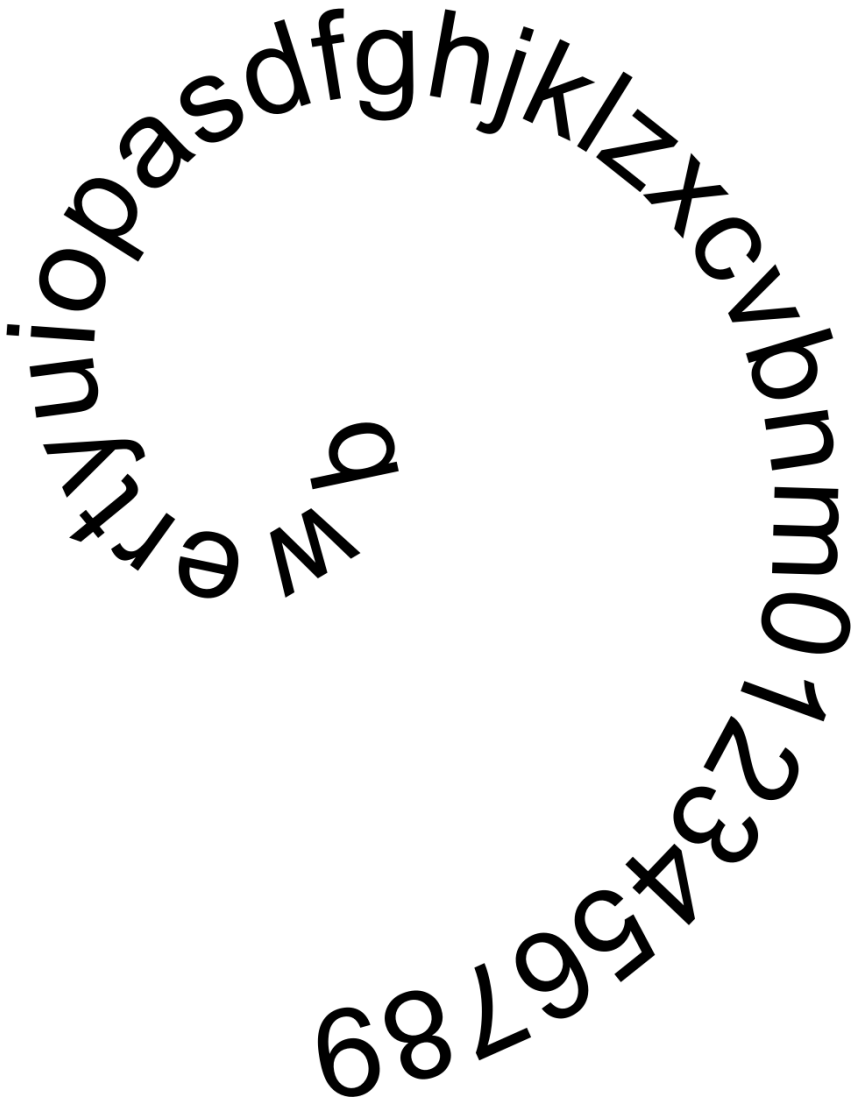

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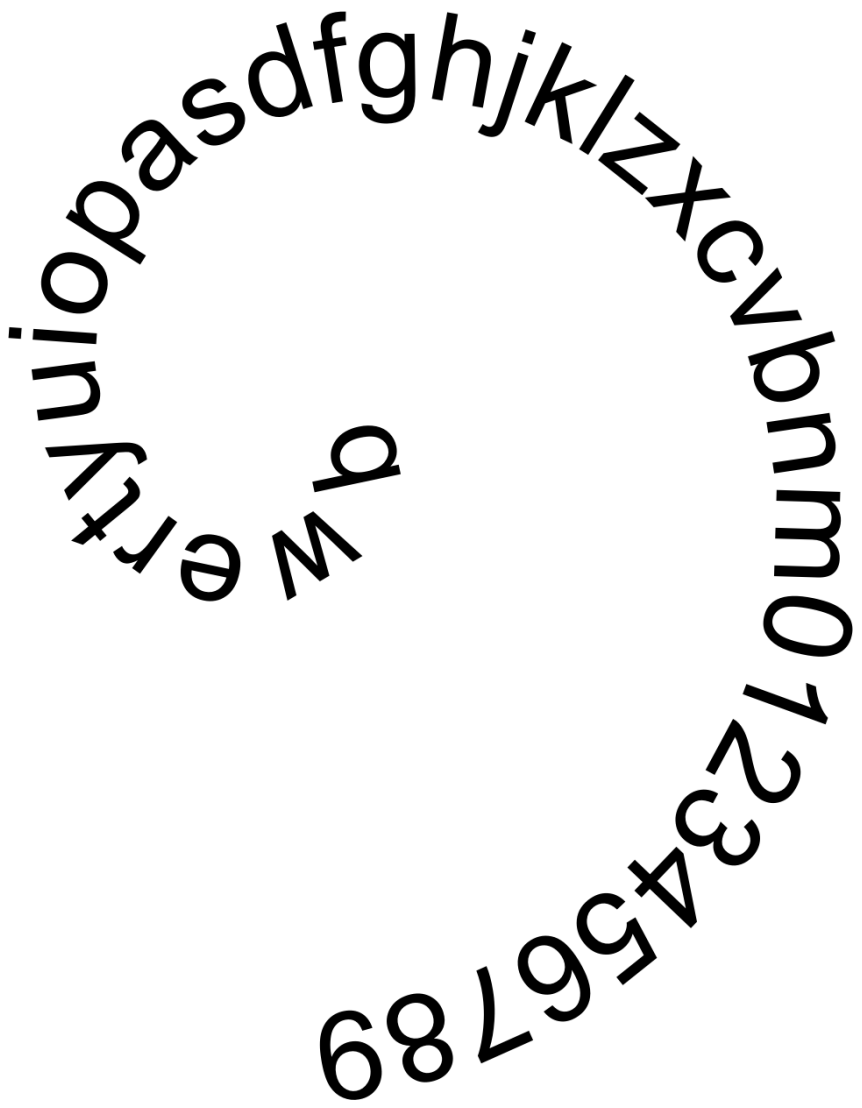

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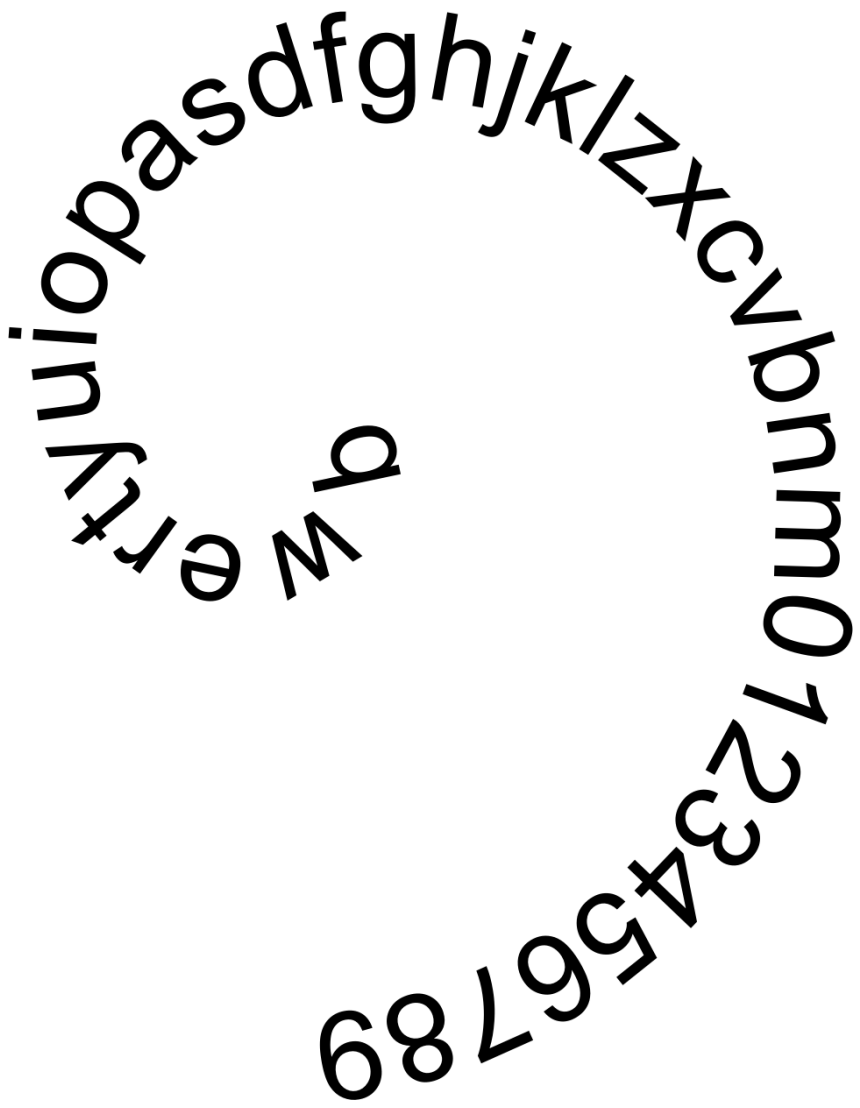

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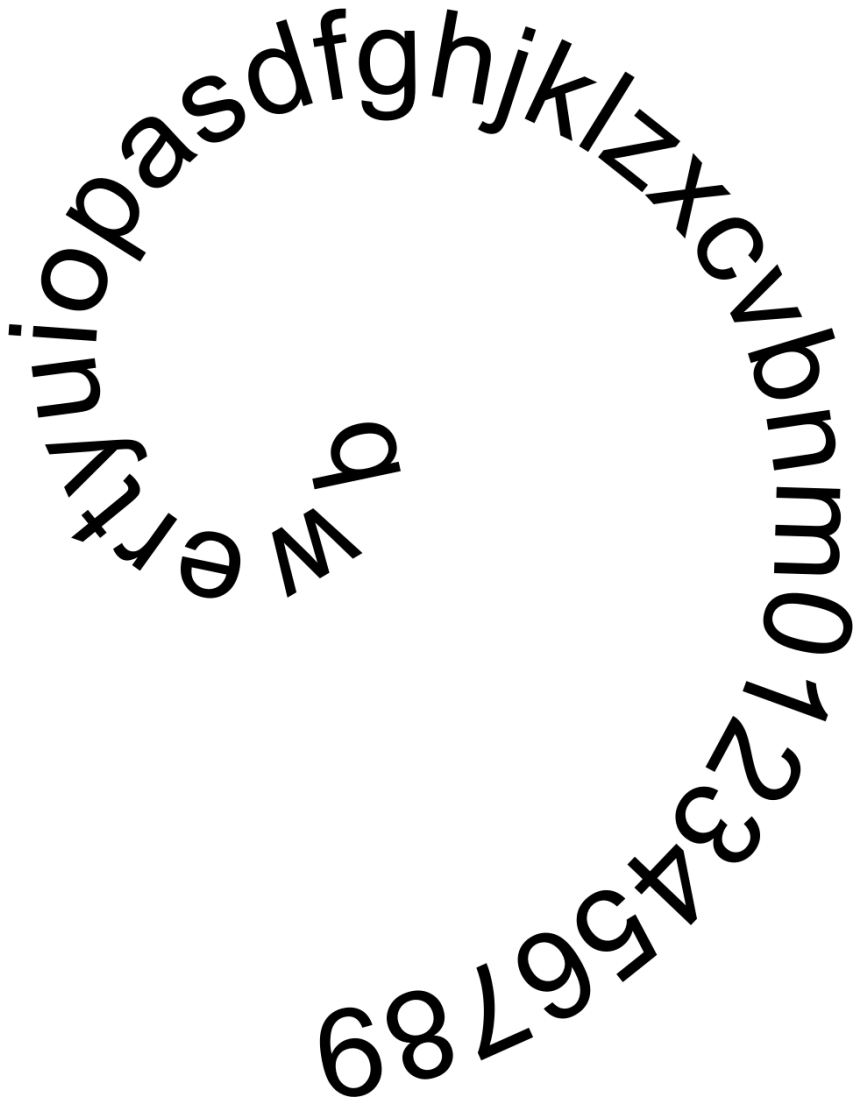

- A p-value is the probability of accepting a hypothesis that is actually false
- In other words, is the probability of a reported difference to be a false positive
- If we perform multiple comparison, a p-value of 0.05 may not be good enough
- We have to account for multiple comparisons

FDR and q-values

- False Discovery Rate (FDR) is a procedure by which we control the maximum number of false positives
- The 'math' bit can be found in Wikipedia

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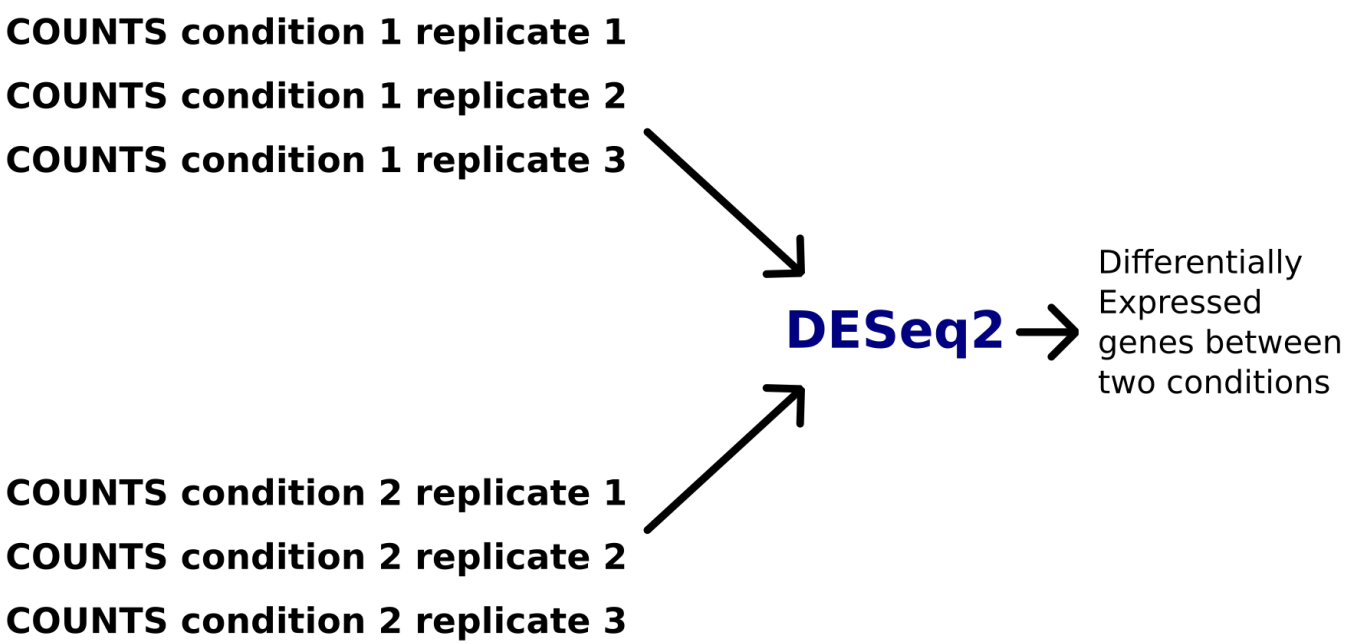
- False Discovery Rate (FDR) is a procedure by which we control the maximum number of false positives
- The 'math' bit can be found in Wikipedia
- A modification of the FDR is the q-value, which is the equivalent to a p-value but for a false discovery rate
- The important thing is that you understand that p-values need to be corrected!

Differential Gene Expression Workflow



Output

- Gene
- log Fold Change (IFC)
- q-value