

RNA-Seq II

Differential Gene Expression

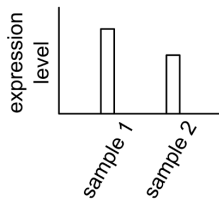
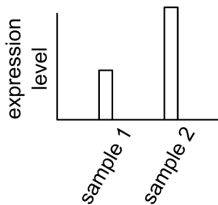
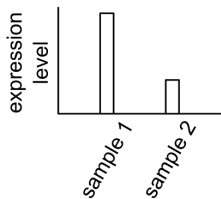
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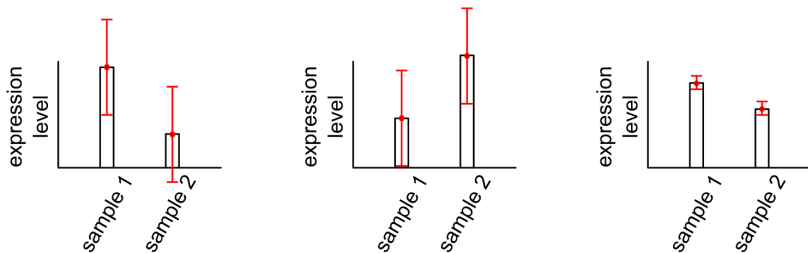
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- 1 Assessing differences
- 2 Multiple comparisons

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

$$V = \frac{1}{n} \sum_{i=1}^n (x_i - \mu)^2 \quad (1)$$

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

To calculate Variances we need multiple measures of each gene:

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

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- For each gene we will have a p-value: the smaller it is, the more significant the difference

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qwertzuiopasdfghjklzxcvbnm0123456789

abcdefghijklmnopqrstuvwxyz
0123456789
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- 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

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- 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

COUNTS condition 1 replicate 1
COUNTS condition 1 replicate 2
COUNTS condition 1 replicate 3

COUNTS condition 2 replicate 1
COUNTS condition 2 replicate 2
COUNTS condition 2 replicate 3

DESeq2

Differentially
Expressed
genes between
two conditions

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