Models And Contrasts In R And Deseq2

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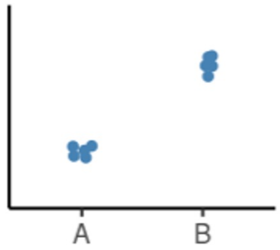
OUTLINE

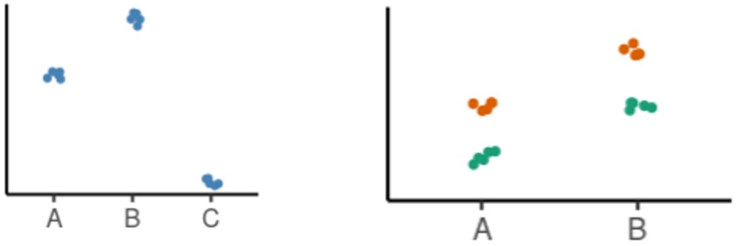
- How to interpret linear models coefficients
    - categorical variables & model matrix



- How to specify models in R using the “formula syntax”

`y ~ x`

- How to interpret the results of different model designs
    - One factor, 3 levels
    - Two factors, additive
    - Two factors, interaction



- How *DESeq2* reports its results and how to interpret them

LINEAR MODEL

A model is a simplified representation of how we think different variables relate to each other.

Linear models are the most commonly used in statistical inference.

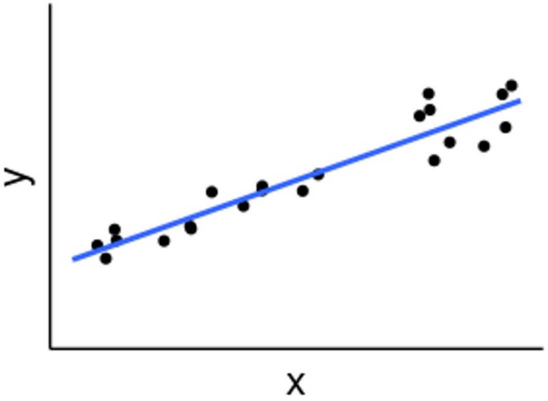
$$\underset{\text{Intercept}}{\beta_0} \quad + \quad \underset{\text{Slope}}{\beta_1} X \quad + \underset{\text{Errors}}{\epsilon}$$

$$Y = \beta_0 + \beta_1 X + \epsilon$$

Intercept Slope Errors

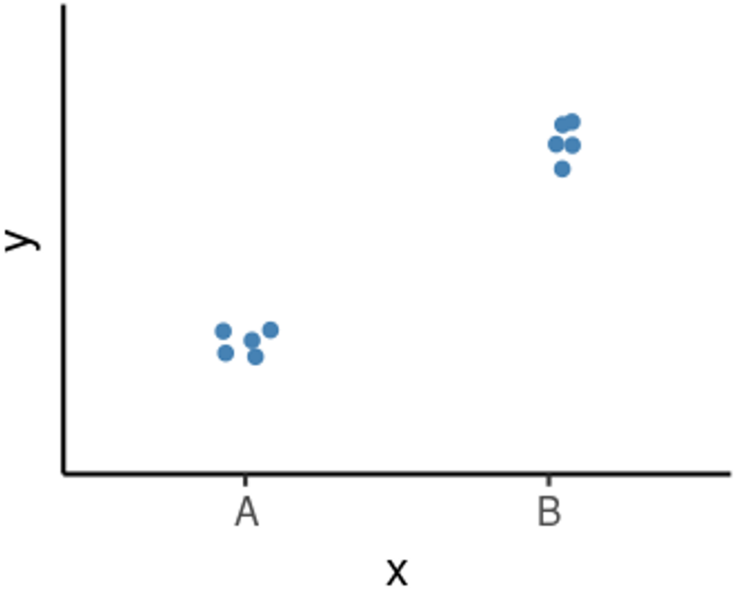
X = Independent variable

Y = Dependent variable



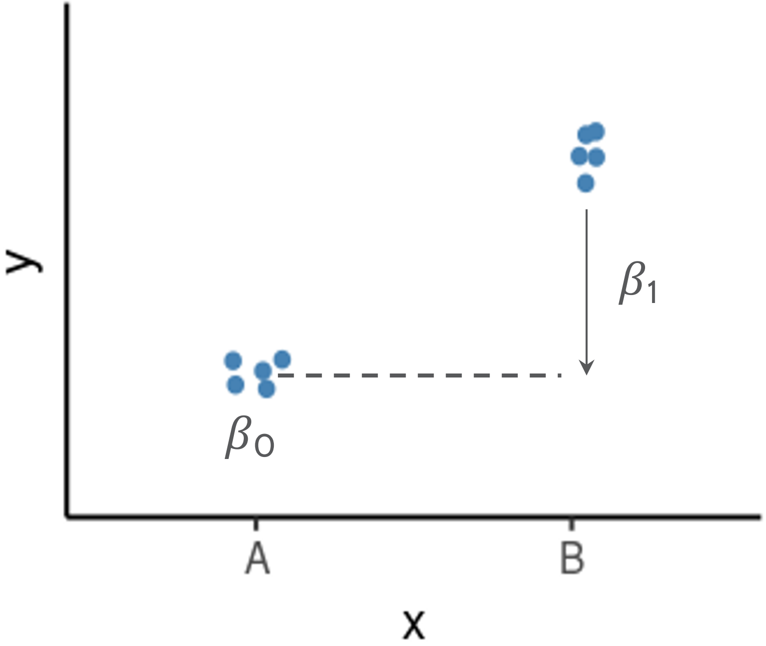
LINEAR MODELS IN R | CATEGORICAL VARIABLES

```
            x
         <factor>
sample1     A
sample2     A
sample3     A
sample4     B
sample5     B
sample6     B
```



LINEAR MODELS IN R | CATEGORICAL VARIABLES

```
              x
          <factor>
sample1     A
sample2     A
sample3     A
sample4     B
sample5     B
sample6     B
```



Model:

$$Y = \beta_0 + \beta_1 X_B + \epsilon$$

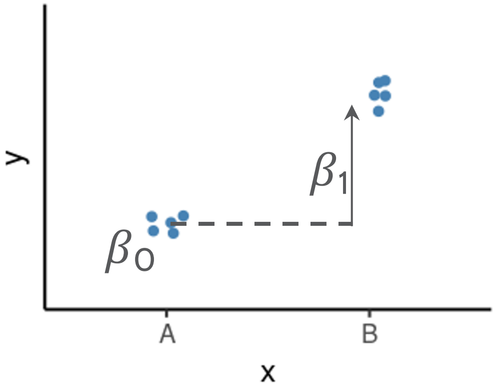
β_0 = *average of the reference group*

β_1 = ***difference*** to the reference group

LINEAR MODELS IN R | CATEGORICAL VARIABLES

```
                x                xB
             <factor>
sample1       A                   0
sample2       A                   0
sample3       A                   0
sample4       B                   1
sample5       B                   1
sample6       B                   1
```

Indicator /
Dummy variable



Model:

$$Y = \beta_0 + \beta_1 X_B + \epsilon$$

β_0 = average of the reference group

β_1 = ***difference*** to the reference group

Example:

$$\beta_0 = 5; \beta_1 = 3$$

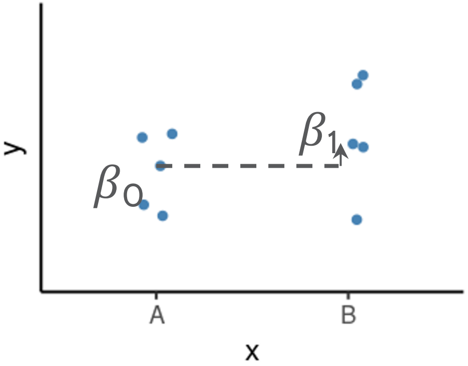
$$Y = 5 + 3 * X_B$$

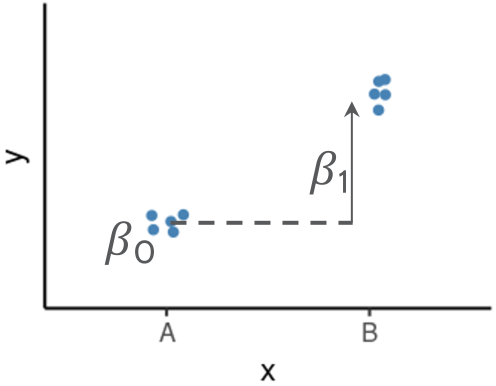
$$Y = 5 + \begin{cases} 3 * \mathbf{0} = 5 \\ 3 * \mathbf{1} = 8 \end{cases} \begin{matrix} \text{if "A"} \\ \text{if "B"} \end{matrix}$$

LINEAR MODELS IN R | NULL HYPOTHESIS TESTING

How compatible is my data with a “boring” hypothesis?

Null hypothesis: $\beta_1 = 0$





Model:

$$Y = \beta_0 + \beta_1 X_B + \epsilon$$

β_0 = *average of the reference group*

β_1 = ***difference*** to the reference group

Test statistic: $\beta_1 / \sigma_{\beta 1}$

(our estimate divided by the uncertainty in that estimate)

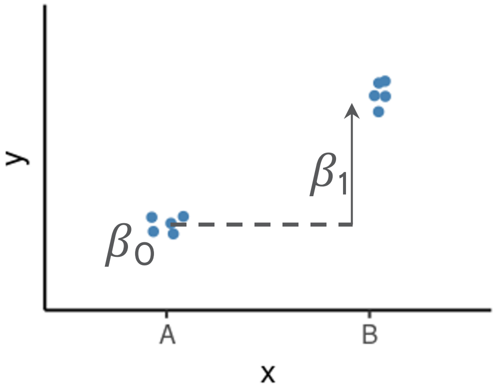
P-value calculated from the test statistic

- Low p-value indicates that the data are not very compatible with the null hypothesis.

Exercise 1

LINEAR MODELS IN R | MODEL SPECIFICATION

```
               x
           <factor>
sample1      A
sample2      A
sample3      A
sample4      B
sample5      B
sample6      B
```



Model:

$$Y = \beta_0 + \beta_1 X_B + \epsilon$$

β_0 = average of the reference group

β_1 = difference to the reference group

Formula syntax in R:

outcome ~ predictors

LINEAR MODELS IN R | MODEL SPECIFICATION

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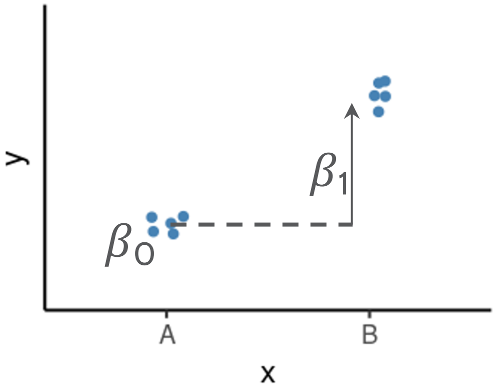


Model:

$$Y = \beta_0 + \beta_1 X_B + \epsilon$$

β_0 = average of the reference group

β_1 = ***difference*** to the reference group

```
              x
          <factor>
sample1       A
sample2       A
sample3       A
sample4       B
sample5       B
sample6       B
```

Design formula → ~ x → Model Matrix

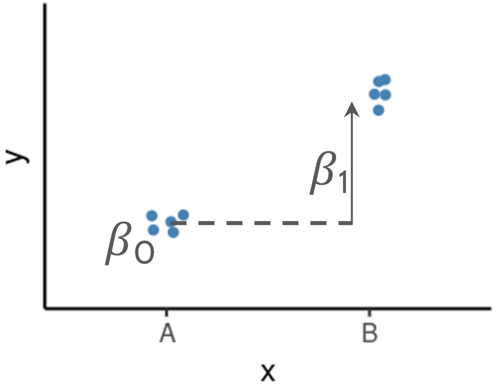
$$\begin{array}{cc} \text{Intercept} & \text{xB} \\ \left(\begin{array}{c} 1 \\ 1 \\ 1 \\ 1 \\ 1 \\ 1 \end{array}\right. & \left.\begin{array}{c} 0 \\ 0 \\ 0 \\ 1 \\ 1 \\ 1 \end{array}\right) \end{array}$$

Example in R worksheet:
“Model Specification - Formula Syntax”

LINEAR MODELS IN R | MODEL SPECIFICATION

Formula syntax in R:

outcome ~ predictors



Model:

$$Y = \beta_0 + \beta_1 X_B$$

β_0 = *average of the reference group*

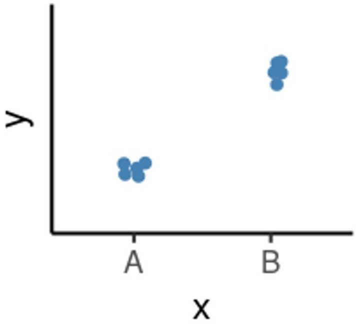
β_1 = ***difference*** to the reference group

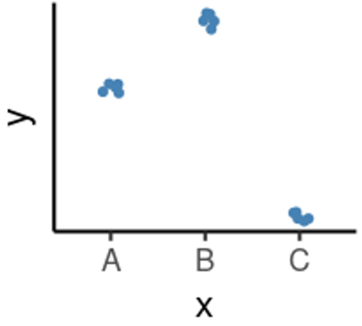
```
              x
        <factor>
sample1   A
sample2   A
sample3   A
sample4   B
sample5   B
sample6   B
```

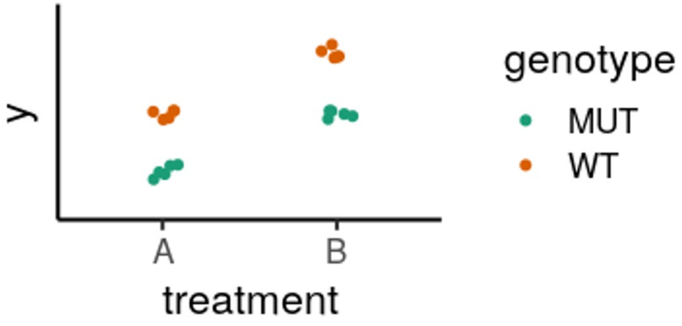
$$\xrightarrow{\text{Design formula}} \sim x \xrightarrow{\text{Model Matrix}}$$

$$\left(\begin{array}{cc} \text{Intercept} & \text{xB} \\ 1 & 0 \\ 1 & 0 \\ 1 & 0 \\ 1 & 1 \\ 1 & 1 \\ 1 & 1 \end{array}\right) \times \begin{pmatrix} \beta_0 \\ \beta_1 \end{pmatrix} = \begin{pmatrix} 1*\beta_0 + 0*\beta_1 \\ 1*\beta_0 + 0*\beta_1 \\ 1*\beta_0 + 0*\beta_1 \\ 1*\beta_0 + 1*\beta_1 \\ 1*\beta_0 + 1*\beta_1 \\ 1*\beta_0 + 1*\beta_1 \end{pmatrix}$$

COMMON DESIGNS


One factor, 2


One factor, 3 levels


Two factors

- Define our model with formula syntax
- Categorical variables are encoded as indicator variables in a model matrix
  - R does this for us
- Interpret coefficients to define hypothesis of interest

COMMON DESIGNS | ONE FACTOR, 3 LEVELS

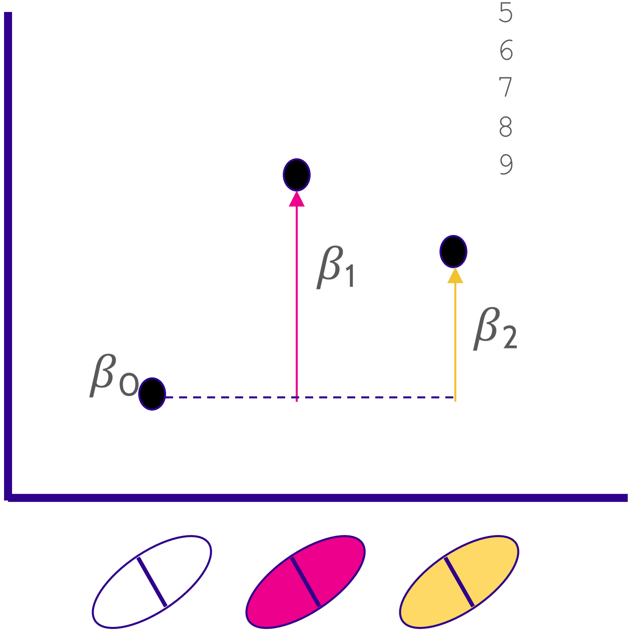
```
        drug
sample1 Pink
sample2 Pink
sample3 Pink
sample4 Yellow
sample5 Yellow
sample6 Yellow
sample7 White
sample8 White
sample9 White
```

Design:

`~ drug`

Model matrix

```
  (Intercept) drugPink drugYellow
1           1        1          0
2           1        1          0
3           1        1          0
4           1        0          1
5           1        0          1
6           1        0          1
7           1        0          0
8           1        0          0
9           1        0          0
```



Null hypothesis:

Pink vs White
$\beta_1 = 0$

Yellow vs White
$\beta_2 = 0$

Yellow vs Pink
$\beta_2 - \beta_1 = 0$

MODEL DESIGNS | TWO FACTORS - ADDITIVE MODEL

```
        drug    genotype
sample1 Pink     WT
sample2 Pink     WT
sample3 Pink     MUT
sample4 Pink     MUT
sample5 White    WT
sample6 White    WT
sample7 White    MUT
sample8 White    MUT
```

Design:

```
~ drug + genotype
```

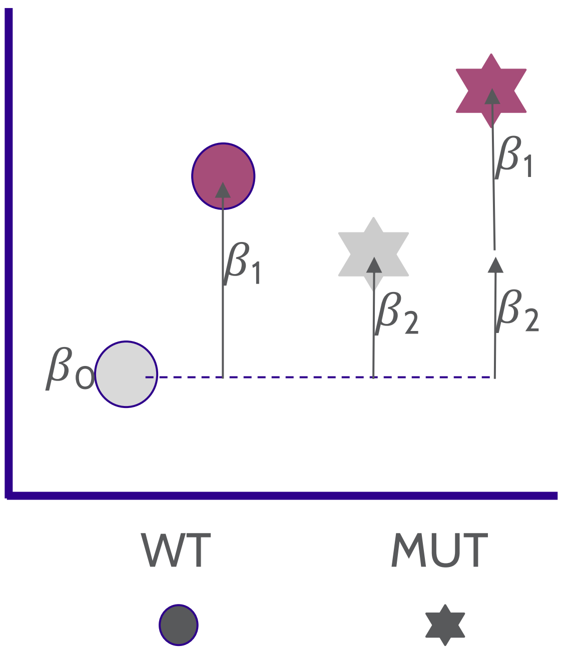
Model Matrix:

```
 (Intercept) drugPink genotypeMUT
1          1        1           0
2          1        1           0
3          1        1           1
4          1        1           1
5          1        0           0
6          1        0           0
7          1        0           1
8          1        0           1
```

Null hypothesis:

Pink vs White drug
$\beta_1 = 0$

WT vs MUT genotype
$\beta_2 = 0$

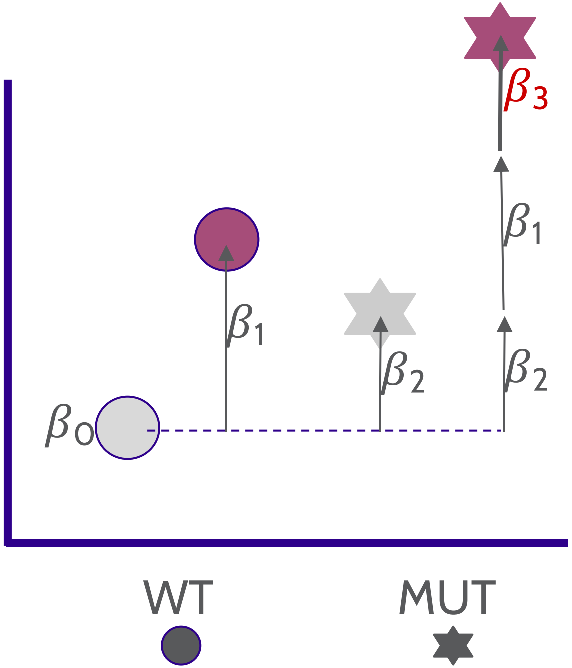


MODEL DESIGNS | TWO FACTORS - INTERACTION MODEL

	drug	**genotype**
sample1	Pink	WT
sample2	Pink	WT
sample3	Pink	MUT
sample4	Pink	MUT
sample5	White	WT
sample6	White	WT
sample7	White	MUT
sample8	White	MUT

Design:

```
~ drug + genotype + drug:genotype
```



Null hypothesis:

Pink vs White (WT)
$\beta_1 = 0$

Pink vs White (MUT)
$\beta_1 + \beta_3 = 0$

WT vs MUT (White)
$\beta_2 = 0$

WT vs MUT (Pink)
$\beta_2 + \beta_3 = 0$

Interaction (“Difference of differences”):
$\beta_3 = 0$

MODEL SPECIFICATION IN *DESEQ2*

- Create DESeqDataSet object
- Add model design:

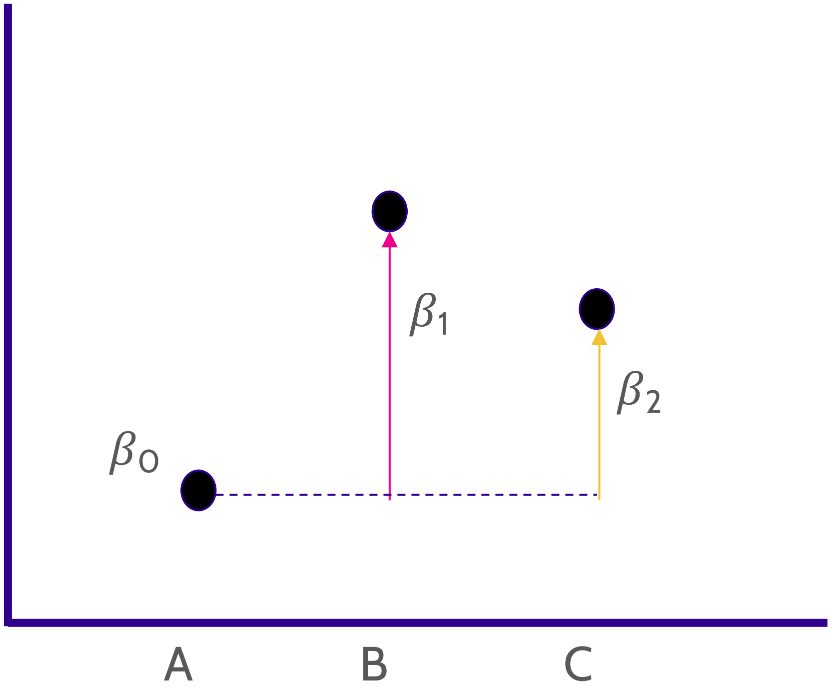
```
design(dds) ← ~ treatment
```

- Fit the statistical model

```
dds ← DESeq(dds)
```

- Check coefficients for hypothesis testing

```
resultsNames(dds)
```



MODEL SPECIFICATION IN *DESEQ2*

- Create DESeqDataSet object
- Add model design:

  `design(dds) ← ~ treatment`

- Fit the statistical model

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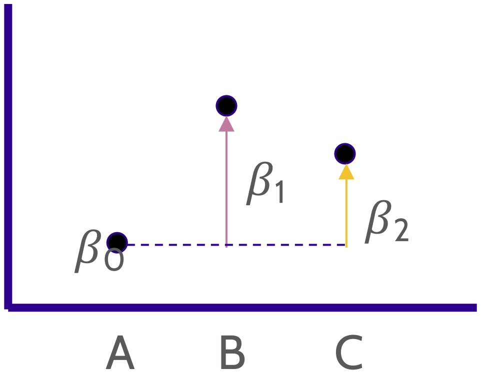
- Check coefficients for hypothesis testing

  `resultsNames(dds)`



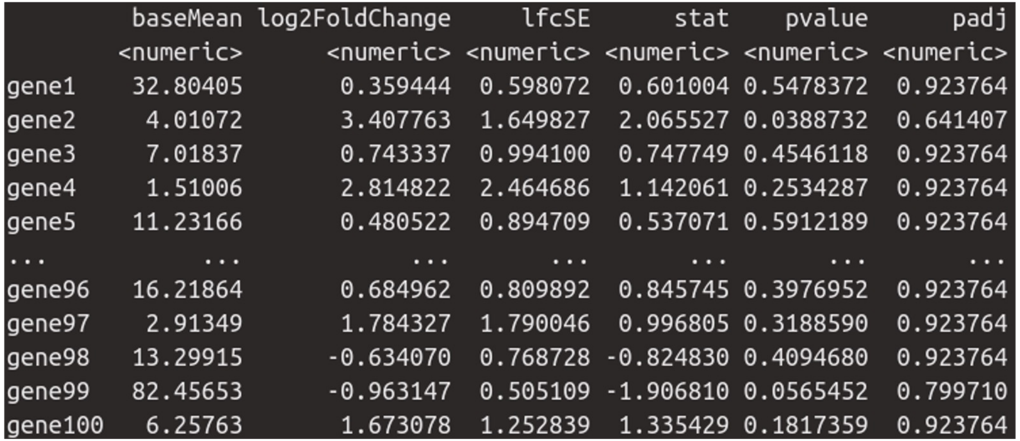
DESeq coefficient names:

$\beta_0 \rightarrow$ Intercept
$\beta_1 \rightarrow$ treatment_B_vs_A
$\beta_2 \rightarrow$ treatment_C_vs_A

	Null Hypothesis
B vs A	$\beta_1 = 0$
C vs A	$\beta_2 = 0$
C vs B	$\beta_2 - \beta_1 = 0$

MODEL SPECIFICATION IN *DESEQ2* | INTERPRETING RESULTS

```
results(dds, contrast = list(“treatment_B_vs_A”))
```



```
         baseMean log2FoldChange     lfcSE      stat    pvalue      padj
        <numeric>      <numeric> <numeric> <numeric> <numeric> <numeric>
gene1    32.80405       0.359444  0.598072  0.601004 0.5478372  0.923764
gene2     4.01072       3.407763  1.649827  2.065527 0.0388732  0.641407
gene3     7.01837       0.743337  0.994100  0.747749 0.4546118  0.923764
gene4     1.51006       2.814822  2.464686  1.142061 0.2534287  0.923764
gene5    11.23166       0.480522  0.894709  0.537071 0.5912189  0.923764
...           ...            ...       ...       ...       ...       ...
gene96   16.21864       0.684962  0.809892  0.845745 0.3976952  0.923764
gene97    2.91349       1.784327  1.790046  0.996805 0.3188590  0.923764
gene98   13.29915      -0.634070  0.768728 -0.824830 0.4094680  0.923764
gene99   82.45653      -0.963147  0.505109 -1.906810 0.0565452  0.799710
gene100   6.25763       1.673078  1.252839  1.335429 0.1817359  0.923764
```

baseMean → Mean across *all* samples

log2FoldChange → log$_2$(B/A) i.e. the difference between treatments

lfcSE → the standard error of the log2FoldChange

stat → the test statistic = log2FoldChange/lfcSE

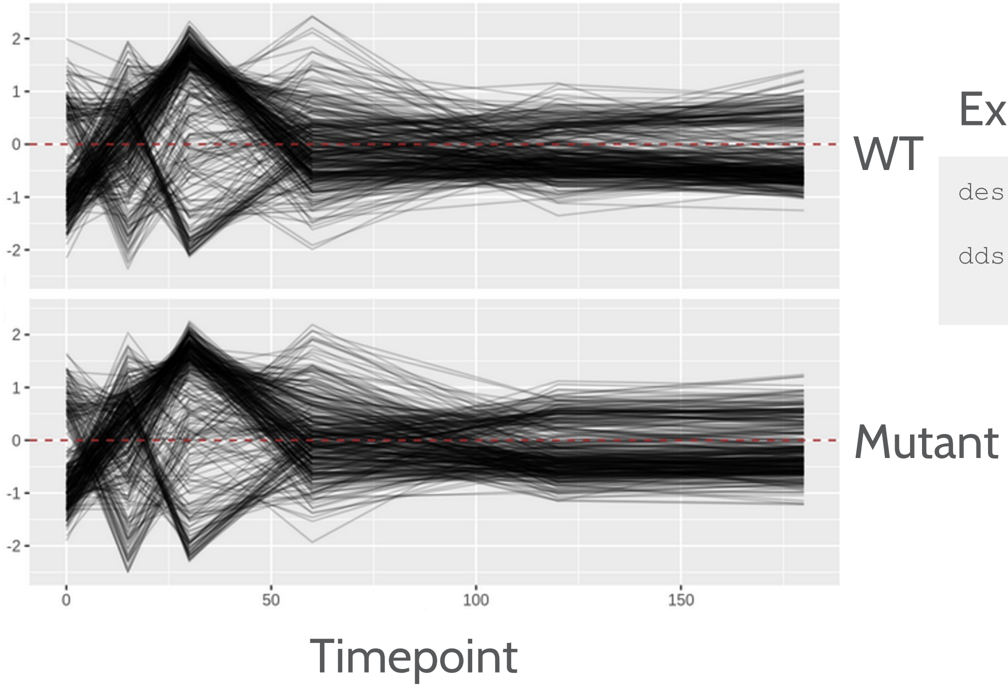
pvalue → the p-value of the Wald test

padj → the p-value adjusted for multiple testing (false discovery rate)

MODEL SPECIFICATION IN *DESEQ2* | LIKELIHOOD-RATIO TEST

The default test in *DESeq2* is the Wald test, testing for null hypothesis that LFC = 0

And alternative is the **Likelihood Ratio Test**



Example:

```
design(dds) ← ~ genotype + timepoint + genotype:timepoint

dds ← DESeq(dds, test = “LRT”,
            reduced = ~ genotype)
```

CONCLUSIONS

- Differential expression tests are based on linear models, where the gene expression is modelled as an outcome of several variables of interest (e.g. treatment, genotype, infection status, etc.).
- Linear models use *indicator or dummy variables* to encode categorical variables in a model matrix.
- To define models in R/DESeq2 we use the formula syntax: `~ variables`
- Some common models are:
  - Single factor: `~ variable1`
  - Two factor, additive: `~ variable1 + variable2`
  - Two factor, interaction: `~ variable1 + variable2 + variable1:variable2`
- Interpreting our model coefficients allows us to define hypothesis/comparisons/contrasts of interest.
- In DESeq2 we use the `results()` function to obtain the log2(fold-change) in gene expression between groups of interest ("contrast").