Models and contrasts in `R/DESeq2`

UNIVERSITY OF CAMBRIDGE

Bioinformatics Training Facility
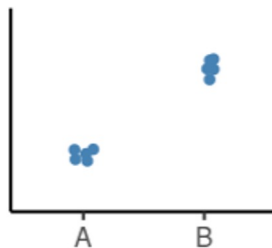
In collaboration with:

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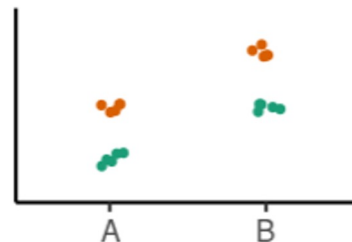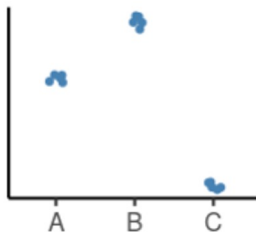
Outline

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

- How to specify models in R using the "formula syntax"

$$y \sim x$$

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

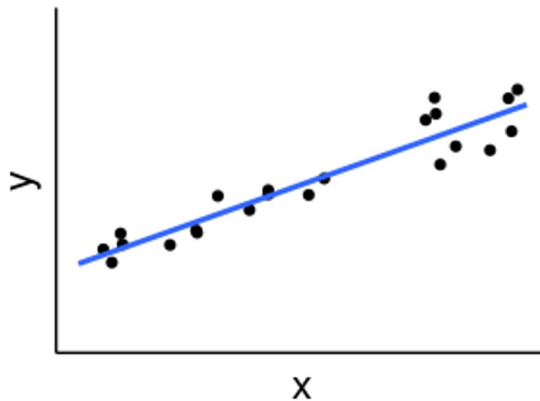- How *DESeq2* reports its results and how to interpret them

Linear Models in R

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

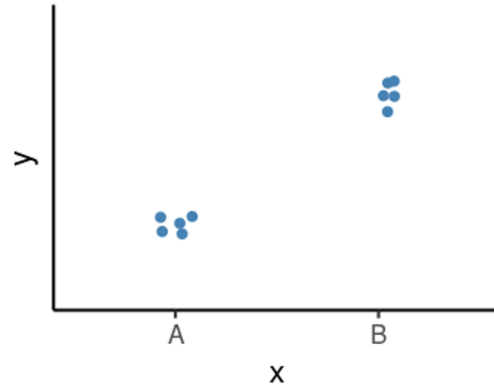**Linear models** are the most commonly used in statistical inference.

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

Intercept   Slope

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

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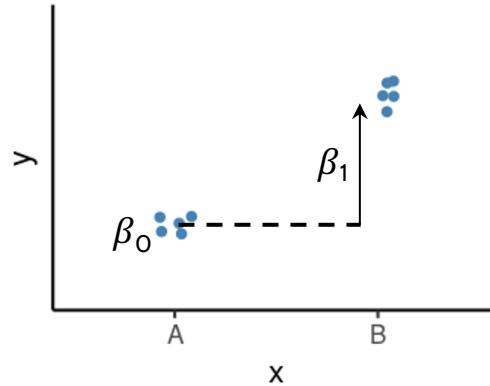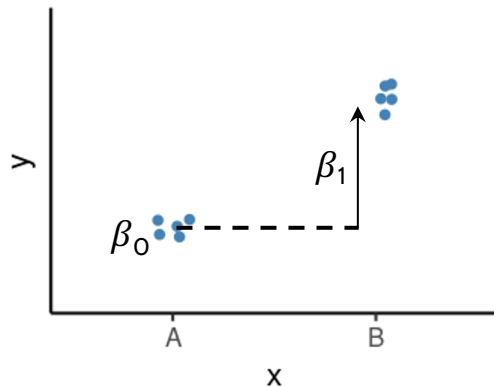

Model:

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

β_0 = *average of the reference group*

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

	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:

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

β_0 = *average of the reference group*
β_1 = ***difference*** to the reference group
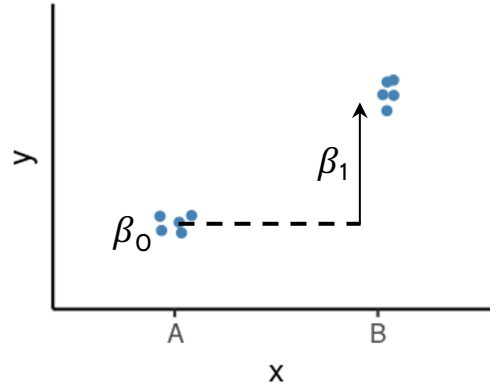
Example:

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

$$Y = 5 + 3 * X_B$$

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

How compatible is my data with a "boring" hypothesis?

Null hypothesis: $\beta_1 = 0$

Model:

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

β_0 = *average of the reference group*
β_1 = **difference** to the reference group

Test statistic: $\beta_1 / \sigma_{\beta1}$

(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.

How compatible is my data with a "boring" hypothesis?

Null hypothesis: $\beta_1 = 0$

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

β_0 = *average of the reference group*
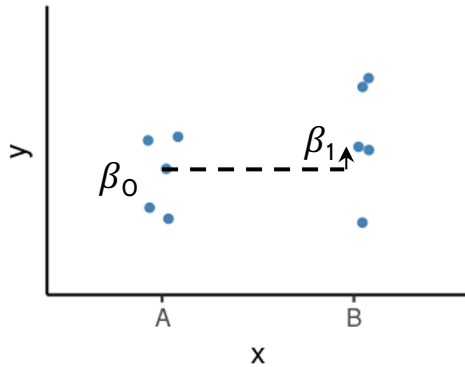β_1 = **difference** to the reference group

Exercise 1

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

(our estimate divided by the uncertainty in that estimate)
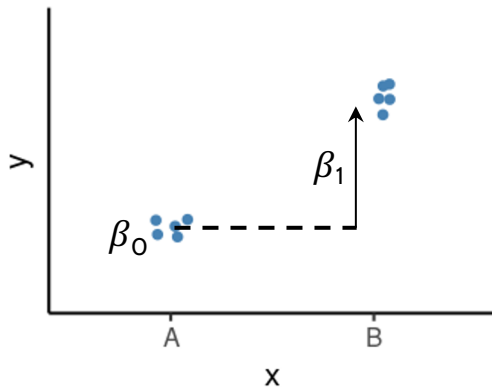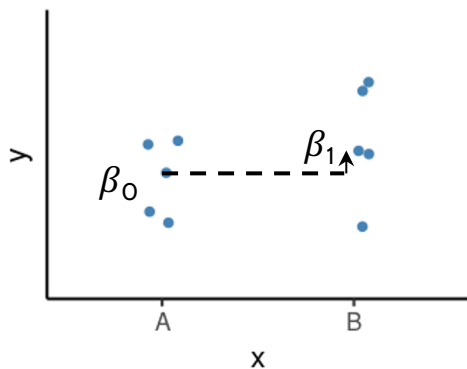
P-value calculated from the test statistic
- Low p-value indicates that the data are not very compatible with the null hypothesis.

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


Model:

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

β_0 = average of the reference group
β_1 = difference to the reference group

Formula syntax in R:

outcome ~ predictors

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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
```

Design formula → ~ x → Model Matrix →

Intercept	xB
1	0
1	0
1	0
1	1
1	1
1	1
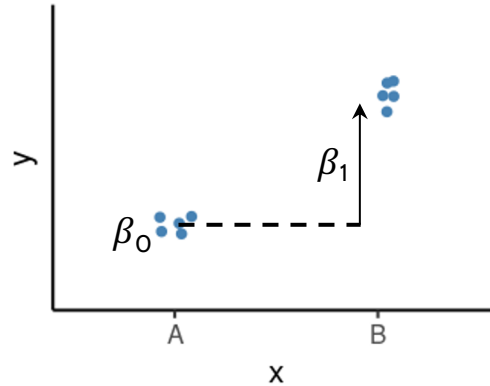
Example in R worksheet:
"Model Specification - Formula Syntax"

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

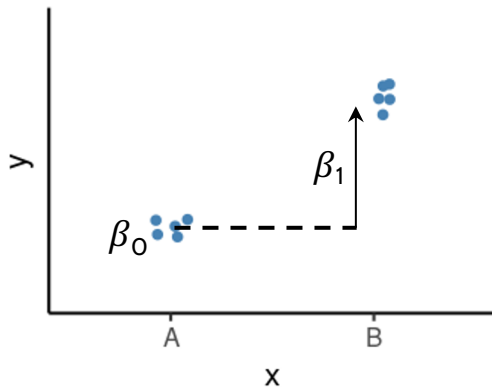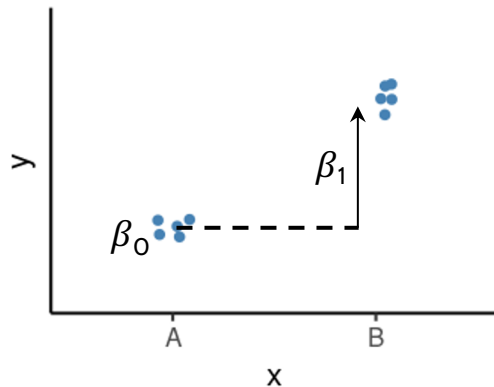y axis, x axis with groups A and B; β_0 at reference, β_1 as difference.

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

Design formula → ~ x → Model Matrix →

Intercept / xB

$$
\begin{pmatrix}
1 & 0 \\
1 & 0 \\
1 & 0 \\
1 & 1 \\
1 & 1 \\
1 & 1
\end{pmatrix}
\times
\begin{pmatrix}
\beta_0 \\
\beta_1
\end{pmatrix}
=
\begin{pmatrix}
1*\beta_0 + 0*\beta_1 \\
1*\beta_0 + 0*\beta_1 \\
1*\beta_0 + 0*\beta_1 \\
1*\beta_0 + 1*\beta_1 \\
1*\beta_0 + 1*\beta_1 \\
1*\beta_0 + 1*\beta_1
\end{pmatrix}
$$

Common Designs

One factor, 2 levels
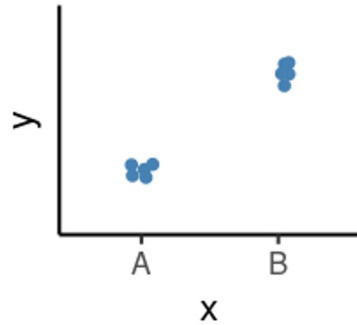
One factor, 3 levels
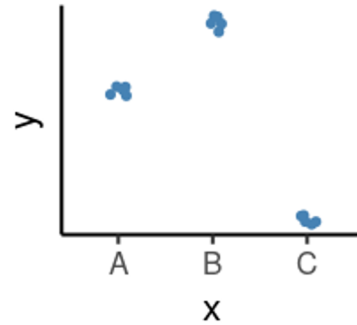
Two factors
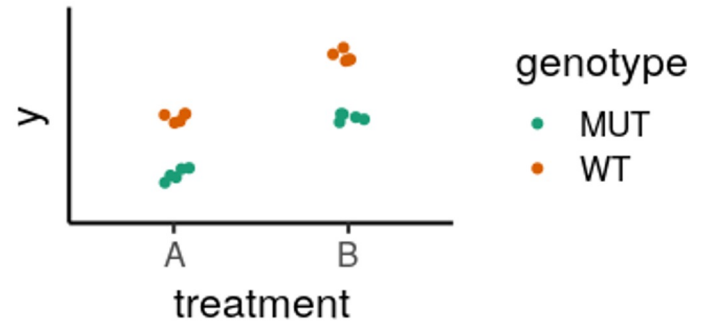
- Define our model with formula syntax

- Categorical variables are encoded as indicator variables in a model matrix

  - R does this for us

- Interpret coefficients to define hypothesis of interest

	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
$\beta_2 - \beta_1 = 0$

$Expr = \beta_0 + \beta_1 drug_{Pink} + \beta_2 drug_{Yellow}$

	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
```
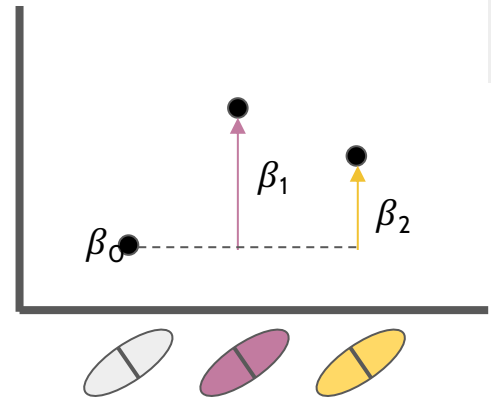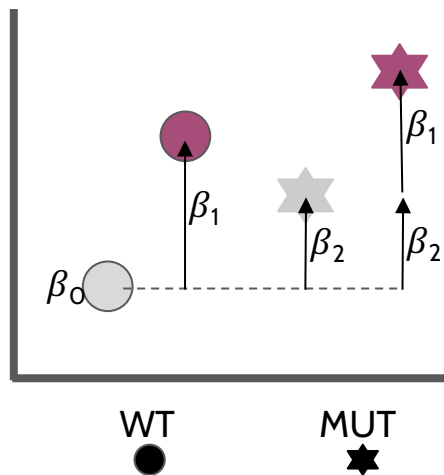
Null hypothesis:

Pink vs White drug
$\beta_1 = 0$

WT vs MUT genotype
$\beta_2 = 0$

$Expr = \beta_0 + \beta_1 drug_{Pink} + \beta_2 genotype_{MUT}$

	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
```

Expr = β_0 + β_1 drug$_{Pink}$ + β_2 genotype$_{MUT}$ + β_3 drug$_{Pink}$genotype$_{MUT}$
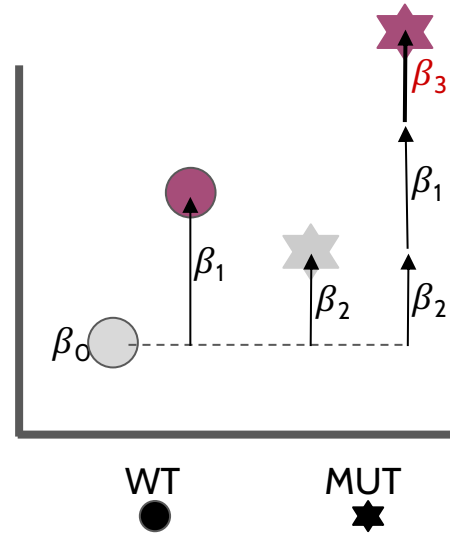
Null hypothesis:

Pink vs White (<u>WT</u>)
β_1 = 0

Pink vs White (<u>MUT</u>)
β_1 + β_3 = 0

WT vs MUT (<u>White</u>)
β_2 = 0

WT vs MUT (<u>Pink</u>)
β_2 + β_3 = 0

Interaction ("Difference of differences"):
β_3 = 0

Model Specification in *DESeq2*

- Create DESeqDataSet object

- Add model design:

  `design(dds) ← ~ treatment`

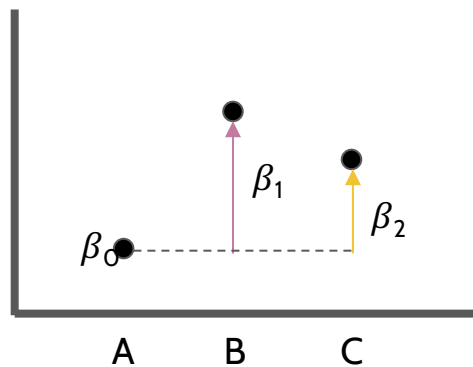- Fit the statistical model

  `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

    ```
    dds ← DESeq(dds)
    ```

- Check coefficients for hypothesis testing

    ```
    resultsNames(dds)
    ```
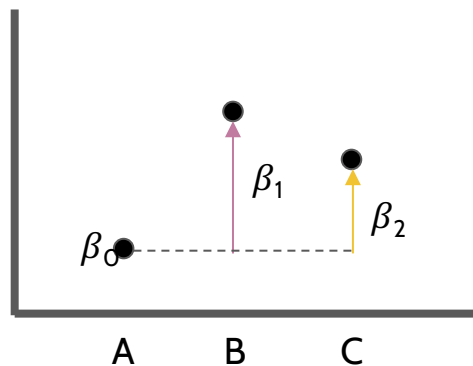

DESeq coefficient names:

$\beta_0 \rightarrow$ 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	$\beta_2 - \beta_1 = 0$

```
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
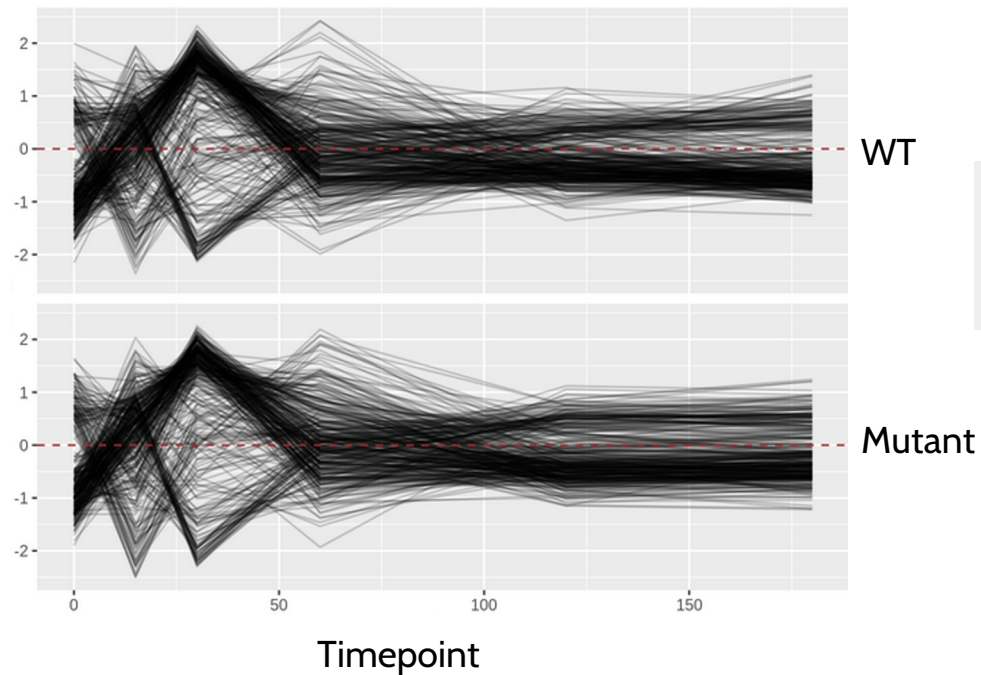
pvalue → the p-value of the Wald test

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

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

And alternative is the **Likelihood Ratio Test**

$$LR = -2ln\left(\frac{L(m_1)}{L(m_2)}\right)$$

WT

Mutant

Timepoint

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").