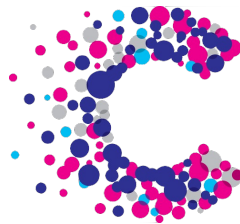


Analysis of RNA-seq data

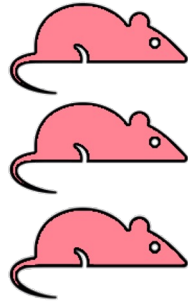
Day 3 recap



Transcriptomic Profiling of Mouse Brain During Acute and Chronic Infections by *Toxoplasma gondii* Oocysts

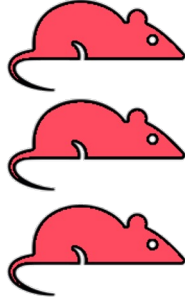
Rui-Si Hu^{1,2}, Jun-Jun He^{1*}, Hany M. Elsheikha³, Yang Zou¹, Muhammad Ehsan¹,
Qiao-Ni Ma¹, Xing-Quan Zhu^{1,4} and Wei Cong^{5*}

"Acute infection"



T. gondii infection
for 11 days

"Chronic infection"

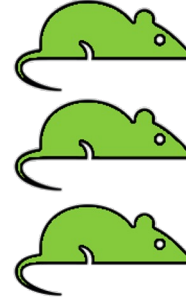


T. gondii infection
for 33 days

Matched control groups



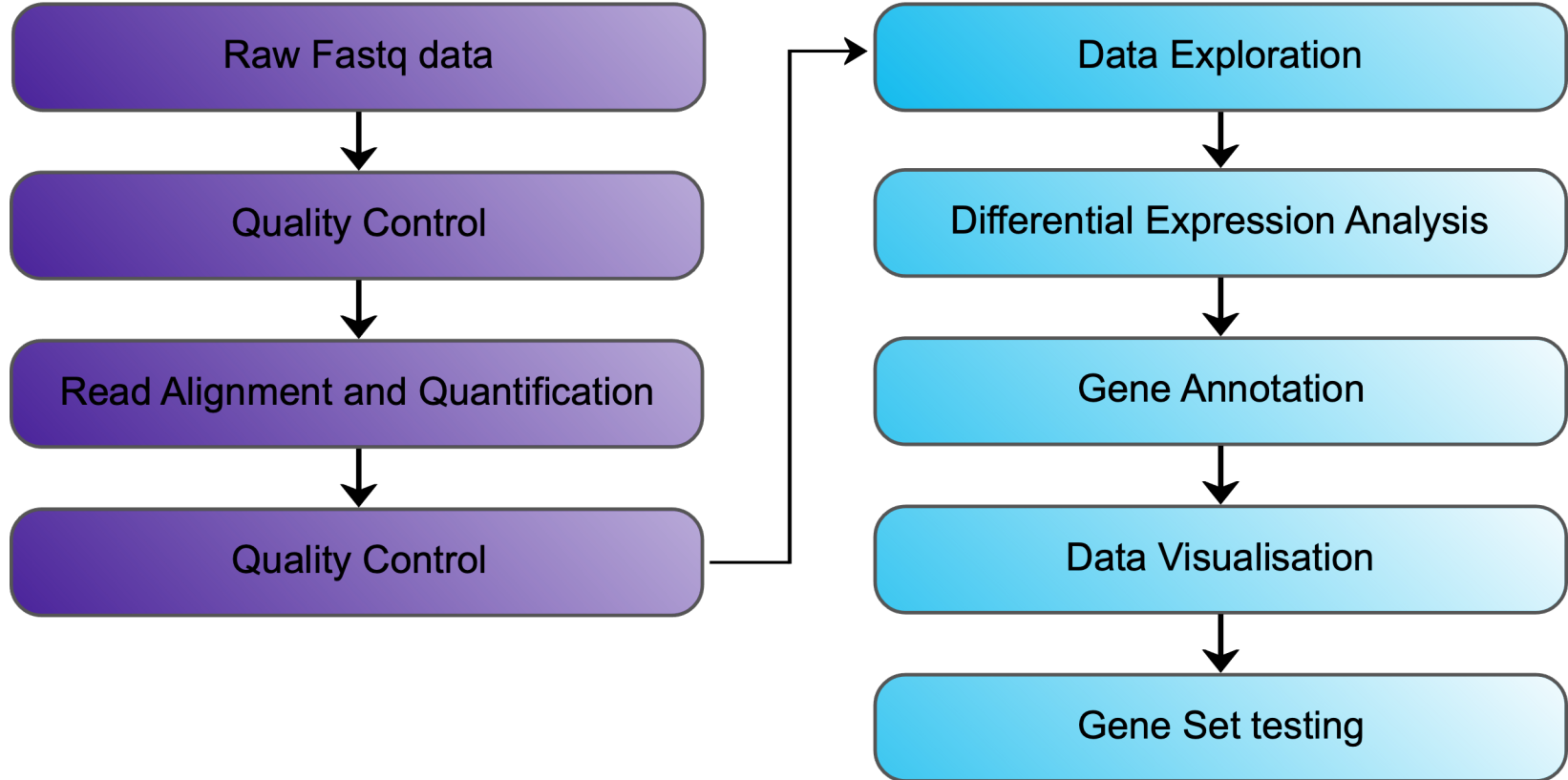
Sham inoculation
for 11 days



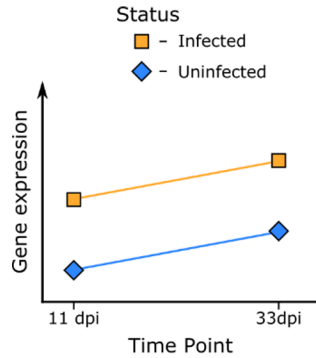
Sham inoculation
for 33 days

RNA-seq

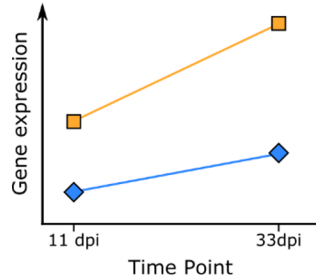
Bioinformatics Analysis Workflow



Two Factor Models



Additive model: gene expression changes with status and time point, but the difference in gene expression between infected and uninfected is the same regardless of the time point



Interaction model: gene expression changes with status and time point, but the difference in gene expression between infected and uninfected changes depending on the time point

DESeq2 | Models and Hypothesis Testing | Two-Factor Additive Model

$$\log_2(q) = \beta_0 + \beta_1 \times Status + \beta_2 \times TimePoint$$

Formula syntax:

`~ Status + TimePoint`

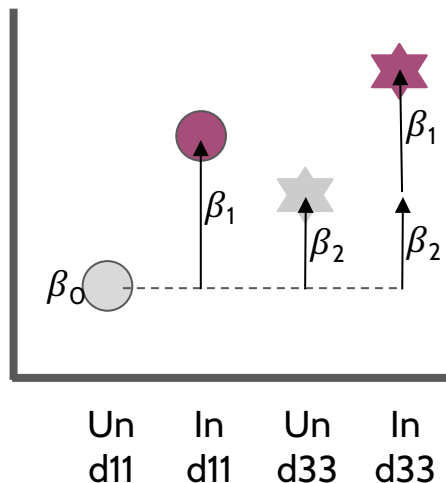
Null hypothesis:

Infected vs Uninfected

$$\beta_1 = 0$$

d33 vs d11

$$\beta_2 = 0$$



DESeq coefficient names:

$\beta_0 \rightarrow$ Intercept

$\beta_1 \rightarrow$

Status_Infected_vs_Uninfected

$\beta_2 \rightarrow$ TimePoint_d33_vs_d11

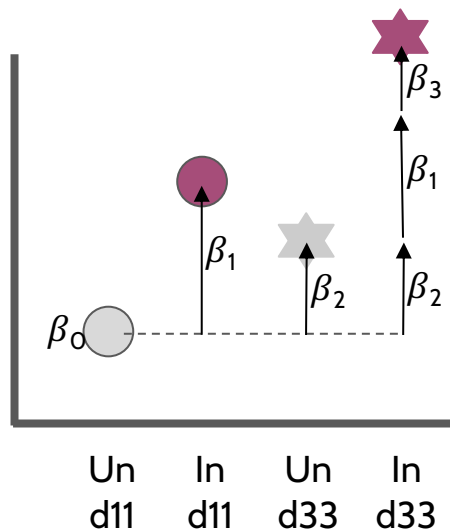
DESeq2 | Models and Hypothesis Testing | **Two-Factor Interaction Model**

$$\log_2(q) = \beta_0 + \beta_1 * Status + \beta_2 * TimePoint + \beta_3 * Status : TimePoint$$

Formula syntax:

```
~ Status + TimePoint + Status:TimePoint
```

```
~ Status * TimePoint
```



Null hypothesis:

Infected vs Uninfected (d11)

$$\beta_1 = 0$$

Infected vs Uninfected (d33)

$$\beta_1 + \beta_3 = 0$$

d33 vs d11 (Uninfected)

$$\beta_2 = 0$$

d33 vs d11 (Infected)

$$\beta_2 + \beta_3 = 0$$

Interaction ("Difference of differences")

$$\beta_3 = 0$$

DESeq2 | Models and Hypothesis Testing

- Considered Experimental Design
- Check your analysis through its process
- Select the simplest appropriate model for your data which best describes the majority of genes
- Know when to visit a bioinformatician or statistician.