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Basic Differential Expression Analysis

Shraddha Pai

Analysis Using R

June 14, 2024

bioinformatics.ca

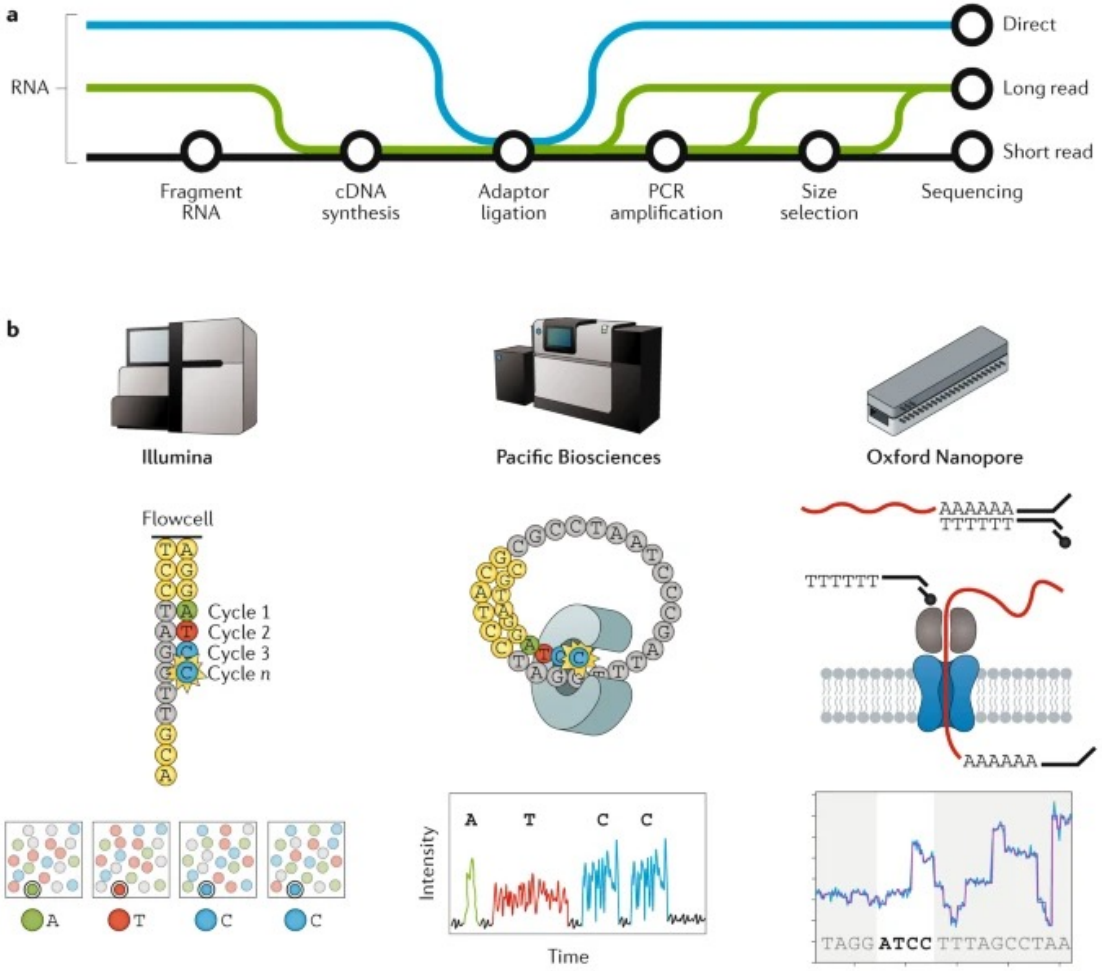
OICR
Ontario Institute
for Cancer Research

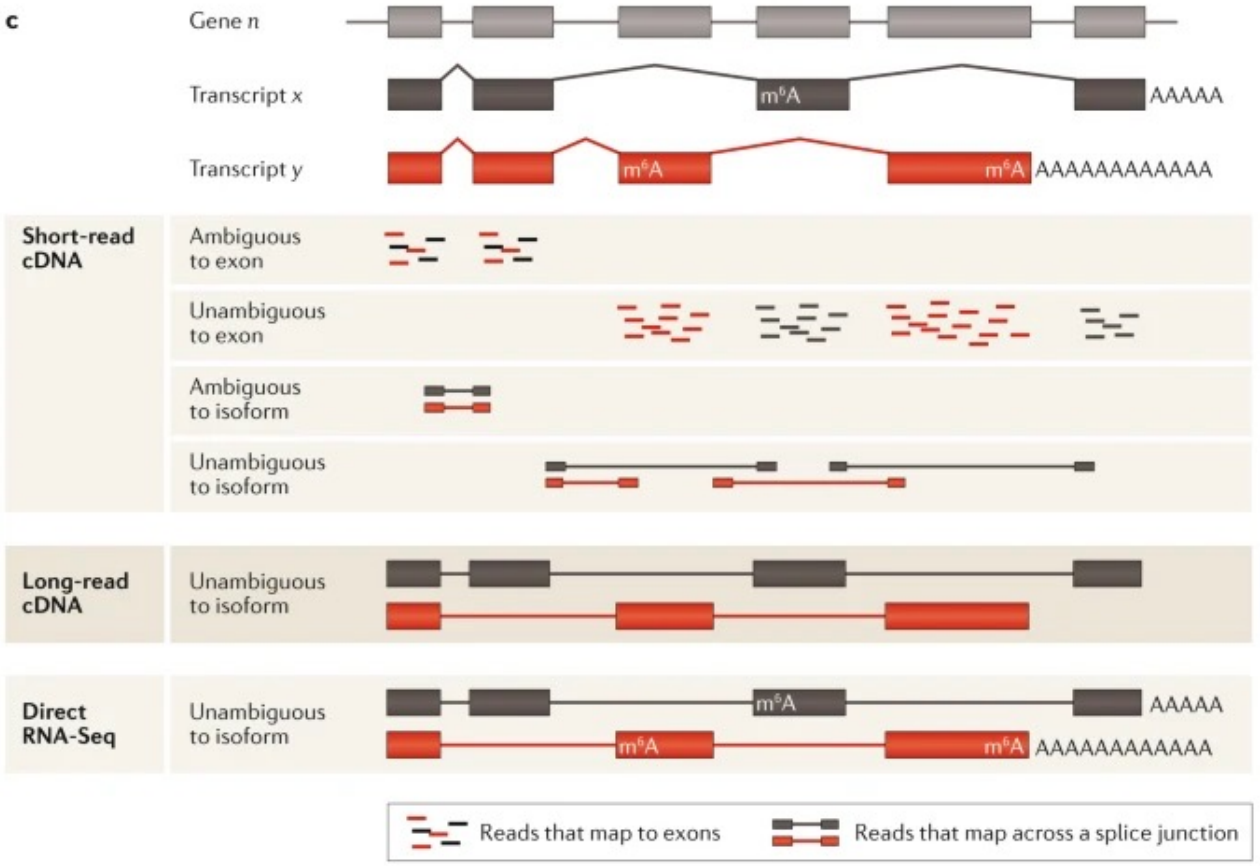
UNIVERSITY OF
TORONTO

Learning Objectives

- By the end of this lecture, you will:
  - Understand the key steps in identifying differentially expressed genes in RNAseq
  - Learn how to use p-value histograms and QQ-plots to gauge how much signal you have after multiple hypothesis testing
  - Learn to create volcano plots to visualize results of differential expression analysis

RNAseq data generation





Stark, Grzelak, Hadfield. (2019). *Nat Rev Gen.*

RNAseq data processing: High-level overview

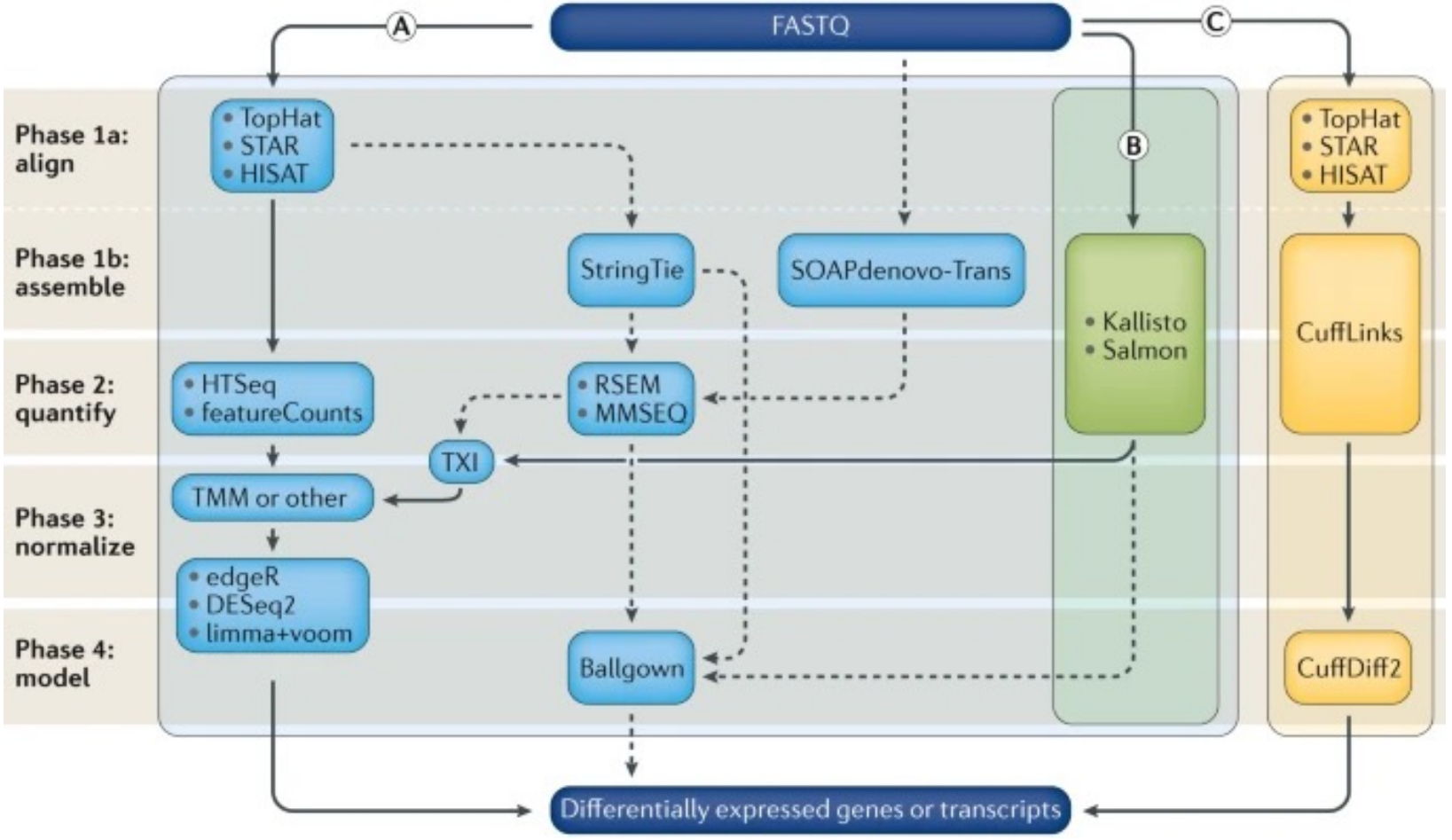
Raw reads

Align reads to genome

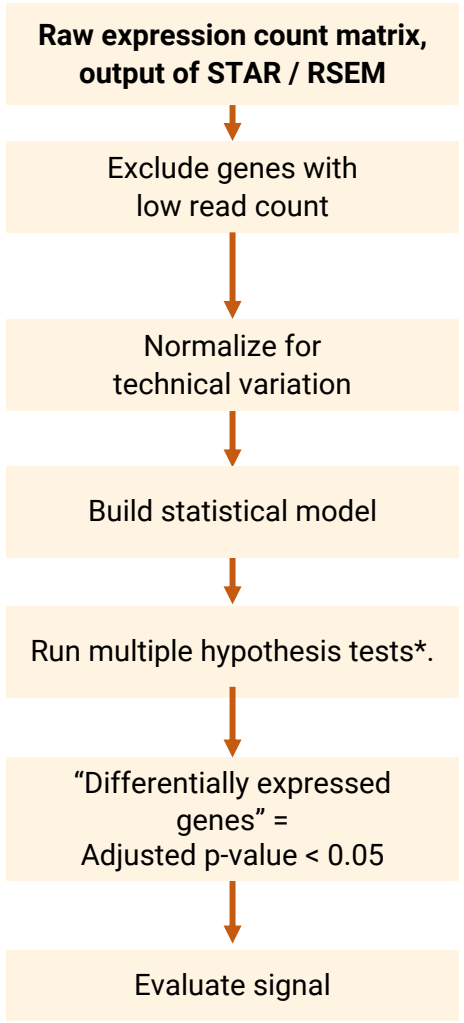
Obtain transcript counts

Normalize

Statistical model for differential gene expression analysis



RNA-seq analysis



~ 20,000 measures (protein-coding genes) → 20,000 individual statistical tests

Multiple testing burden

20,000 p-values

Reduce multiple testing burden

Variation in sequencing depth

$log(xpr) = \beta_0 + \epsilon$* *null model*

$log(xpr) = \beta_0 + \beta_1(disease) + \epsilon$ *full model*

} Likelihood ratio test (LRT)

Negative binomial distribution *(class of generalized linear models)*

Signal in count data

Correct for multiple tests

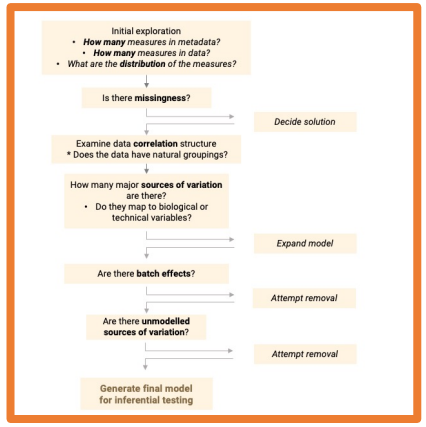
* $\epsilon \sim log(Normal)$

Let’s look at a worked example for RNAseq analysis.

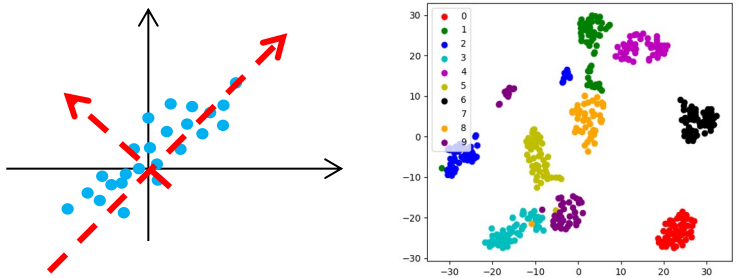
Exercise time.

Recap course

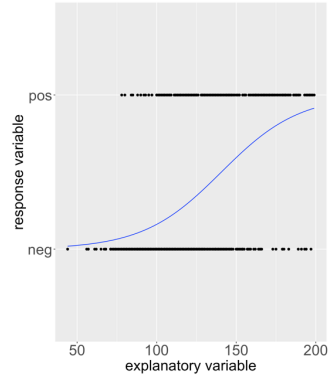
Module 1: Systematic **exploratory data analysis**



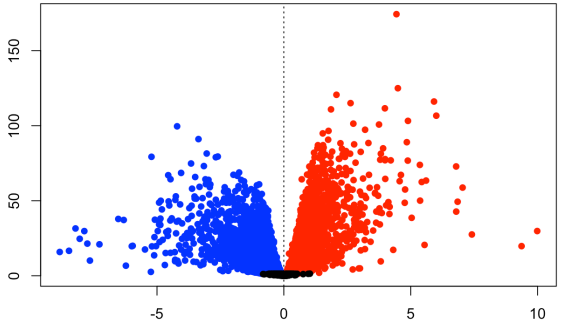
Module 2: **Dimensionality reduction** to identify major sources of variation in your data



Module 3: **Generalized linear models** to fit binary response variables (and RNAseq data!)



Module 4: **Differential expression analysis**, multiple hypothesis testing



Enjoy exploring your data!

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