RNA-Seq

Experimental design, analysis and interpretation

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RNA-seq
Experimental design considerations

What are my aims and what is my hypothesis?
How will RNA-seq allow me to test my hypothesis?

What is the integrity of my RNA (RIN)?
How much RNA do I have?

What type(s) of RNA do I want to measure?

- Total RNA
- PolyA transcripts
- Small RNA (i.e. miRNA)
- Non-coding RNA
- Splice variants
- Anti-sense transcripts

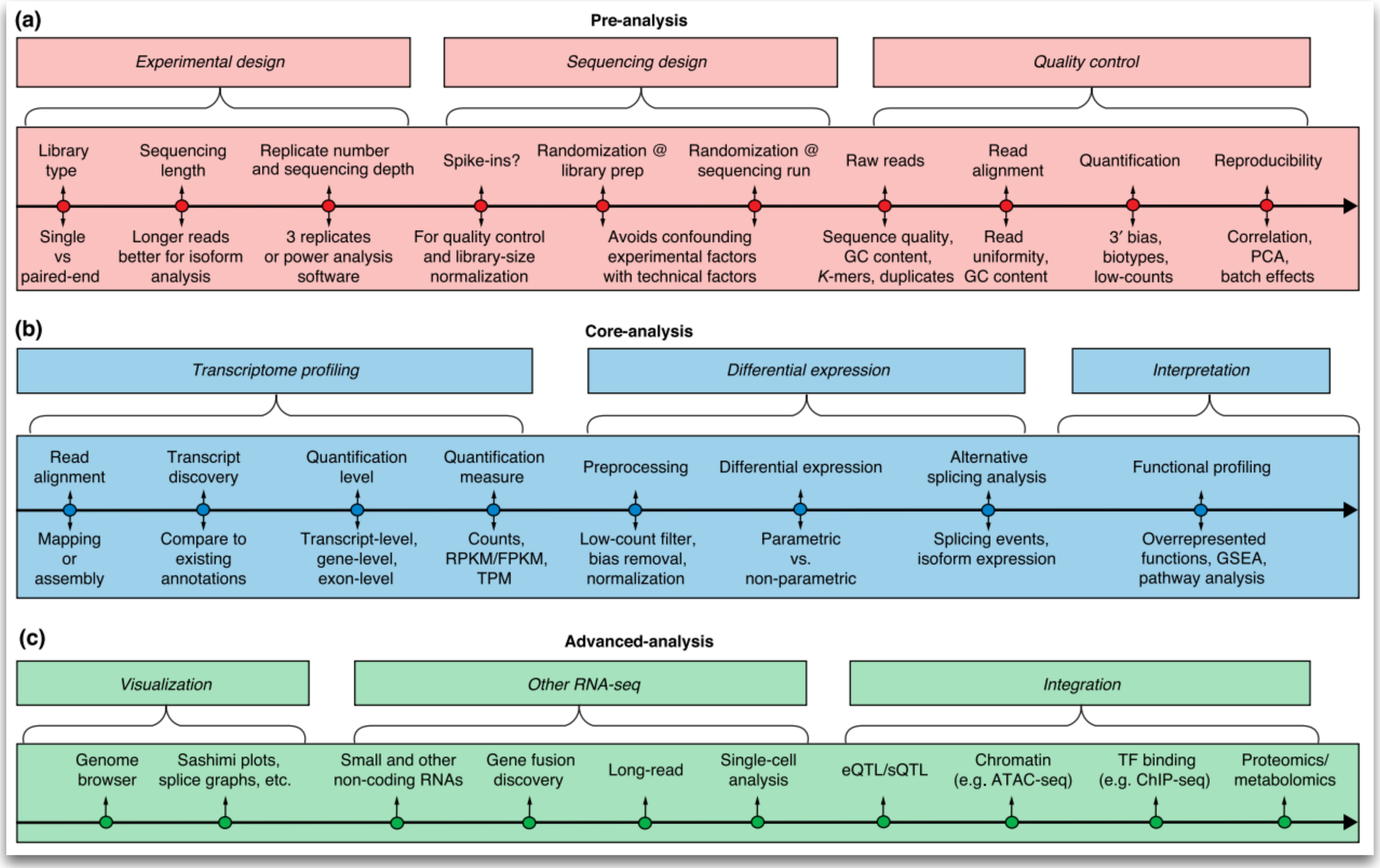
Details like this help select the RNA-seq library preparation method.

RNA-seq
Experimental design considerations

- Does the organism under study have a good reference genome/transcriptome assembly?
- Do I need to perform a *de novo* transcriptome assembly
- Am I trying to detect and differentially test genes with low expression levels?
- How deep should I sequence each library?
- Will my samples be processed in batches, and if so, how will I control for batch effects?

What are other things you might consider?

A generic roadmap of RNA-seq analyses



Conesa *et. al.* A survey of best practices for RNA-seq data analysis. Genome Biology, 17:13 (2016)

Example dataset

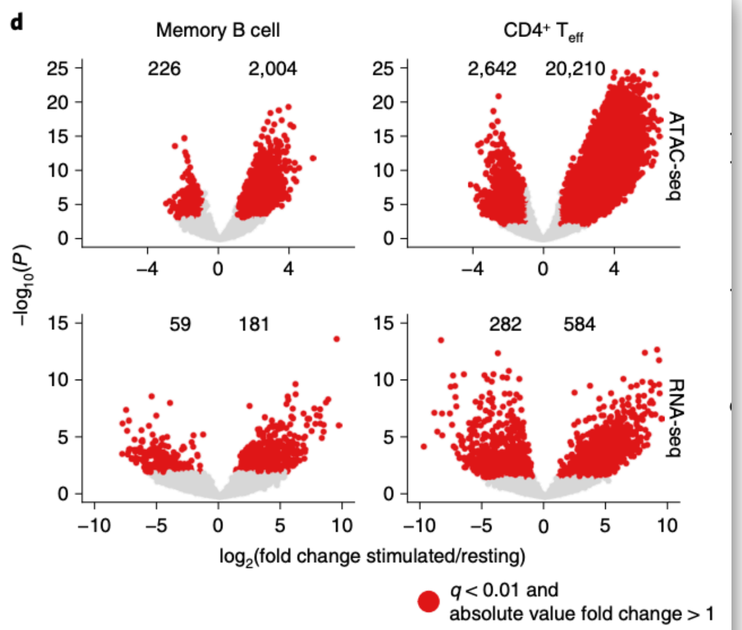
RNA-seq
Human CD4+ T-cells: Unstimulated vs Stimulated

In the first two sections, we will just be working with chromosome 22 data from 4 libraries to enable efficient processing

ARTICLES
https://doi.org/10.1038/s41588-019-0505-9
nature genetics

Landscape of stimulation-responsive chromatin across diverse human immune cells

Diego Calderon[1,18], Michelle L. T. Nguyen[2,3,18], Anja Mezger[4,5,18], Arwa Kathiria[4], Fabian Müller[4], Vinh Nguyen[3], Ninnia Lescano[3], Beijing Wu[4], John Trombetta[4], Jessica V. Ribado[4], David A. Knowles[4,6], Ziyue Gao[4,7], Franziska Blaeschke[2,3,8], Audrey V. Parent[3], Trevor D. Burt[9,10], Mark S. Anderson[3], Lindsey A. Criswell[11,19*], William J. Greenleaf[4,12,13,19*], Alexander Marson[2,3,8,11,13,14,15,16,19*] and Jonathan K. Pritchard[4,7,17,19*]



Getting started

1. This workshop is designed to demonstrate a few ways RNA-seq data can be easily analysed in-depth primarily using R packages.

2. There are many ways to approach RNA-seq analysis, and I encourage you to explore more than what is presented here

3. If possible, work in pairs or in small groups through these exercises and explore how you could modify the analyses.

Getting started

1. **Open RStudio** on your own computer or navigate to http://35.226.108.244:8787 to use a virtual machine server with RStudio

2. Within RStudio, select File > New project and then click ‘Version control’, then click ‘Git’.

3. In the Repository URL box, enter https://github.com/SamBuckberry/RNAseq-workshop.git