

Introduction to Transcriptomics

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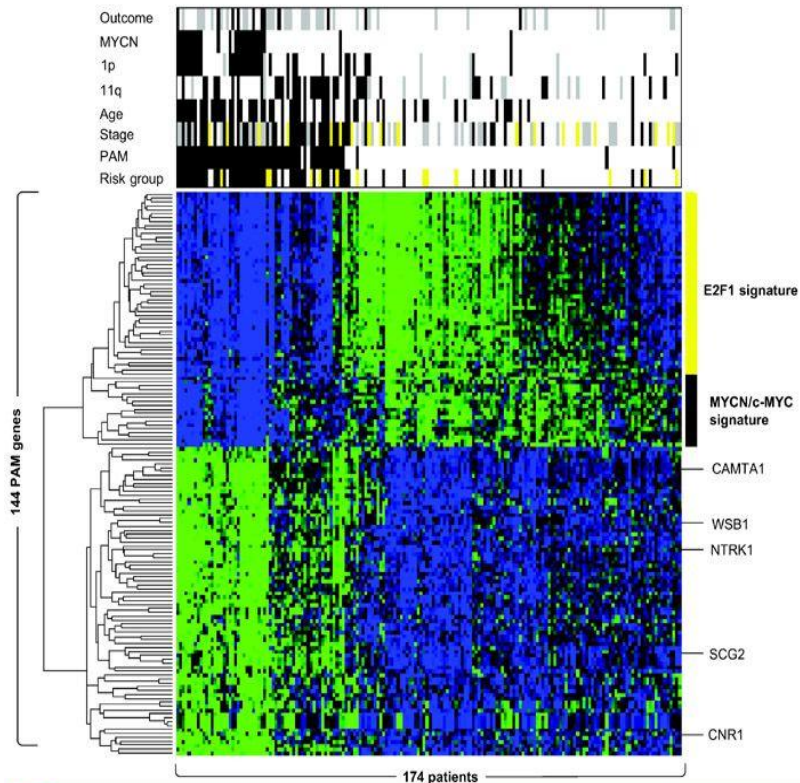
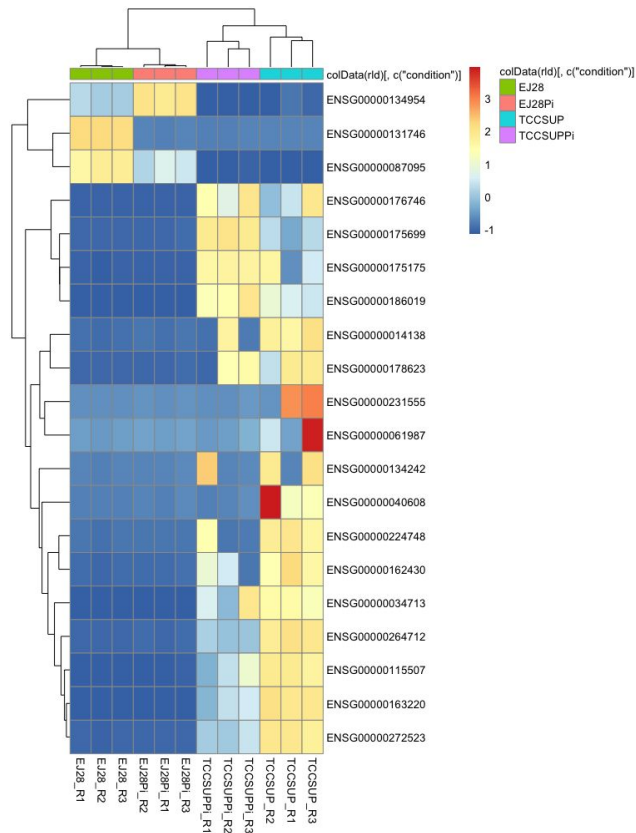
June 25, 2021

 @babasaraki1



Transcriptomes give us information of gene expression

Identify genes differentially expressed, identify functional changes...



Medscape

Source: Future Oncol © 2009 Future Medicine Ltd

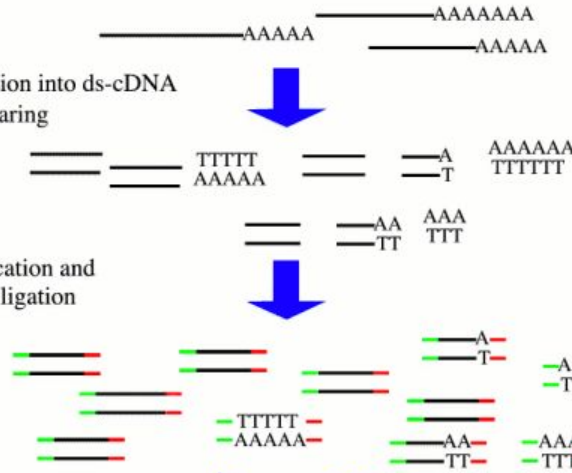
Overview of RNA-Seq

Transcriptome profiling using NGS

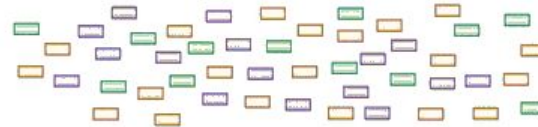
extraction of poly-A RNAs

conversion into ds-cDNA
and shearing

amplification and
adapter ligation

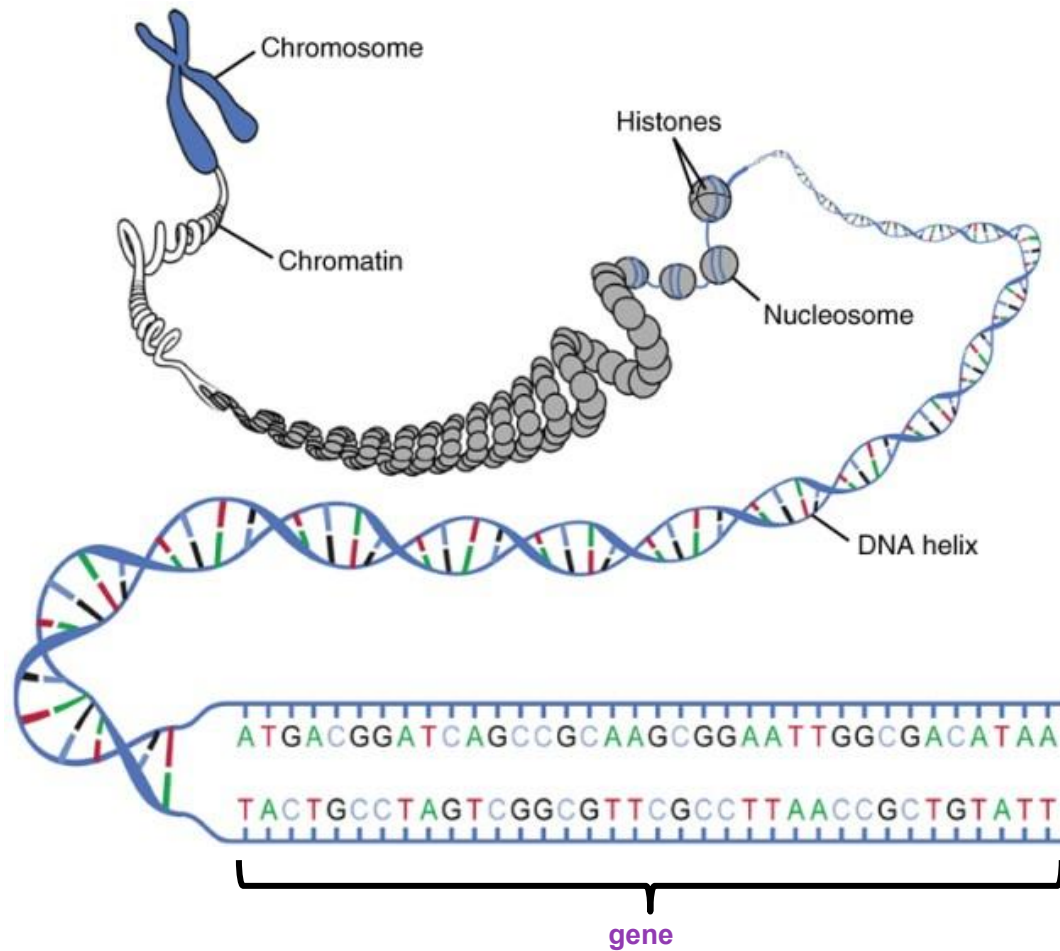


RNA-Seq reads

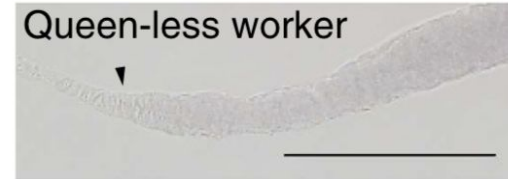
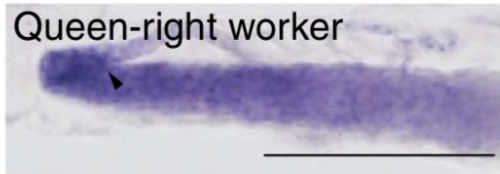
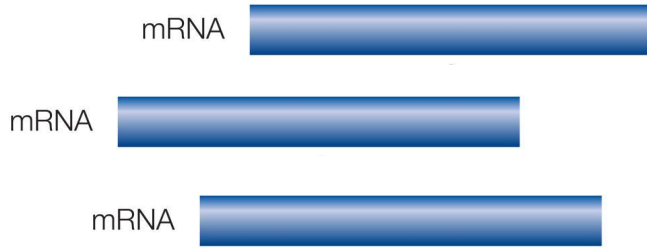


Why transcriptomes in biological research?

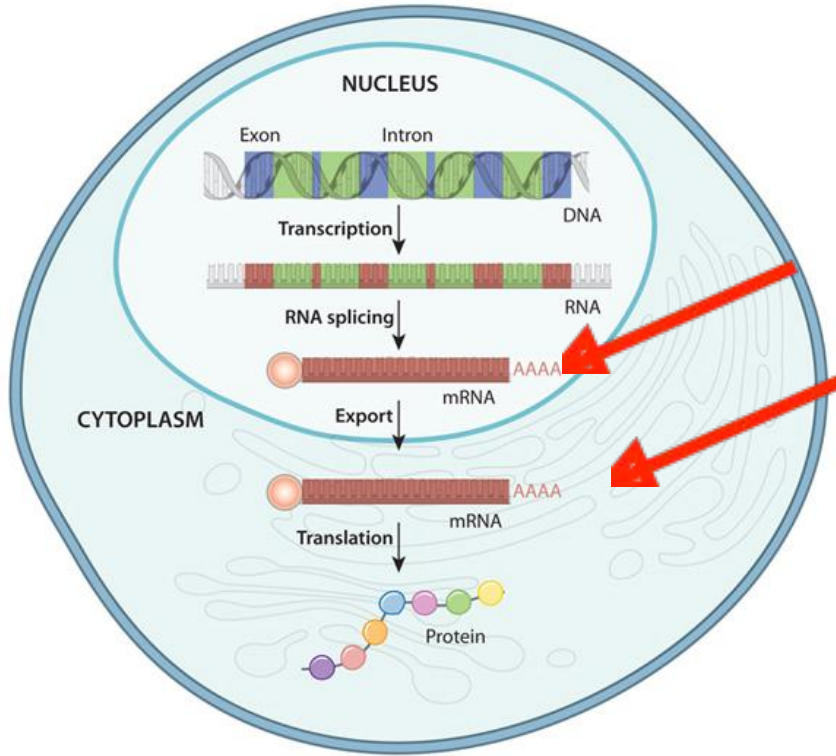
Pros	Cons
Easy, accessible way to see and quantify gene expression	Snapshot in time (different times, different expression patterns)
Immediate access to the protein coding portion of the genome	Difficult to ensure that you have sampled a single cell type.
Identify alternative splicing	Absence of a gene does not mean it is not present in the genome.
Identify Single Nucleotide Polymorphisms (SNPs) in coding regions	Statistical analysis is highly dependent on experimental design.



Gene expression = transcript abundance



Stages of gene expression



RNA-Seq captures the mature messenger RNA (mRNA)

Targets the characteristic poly-A tail of the mRNA

The assumption is that the amount of mRNA for any gene is reflective of its impact on the cell function

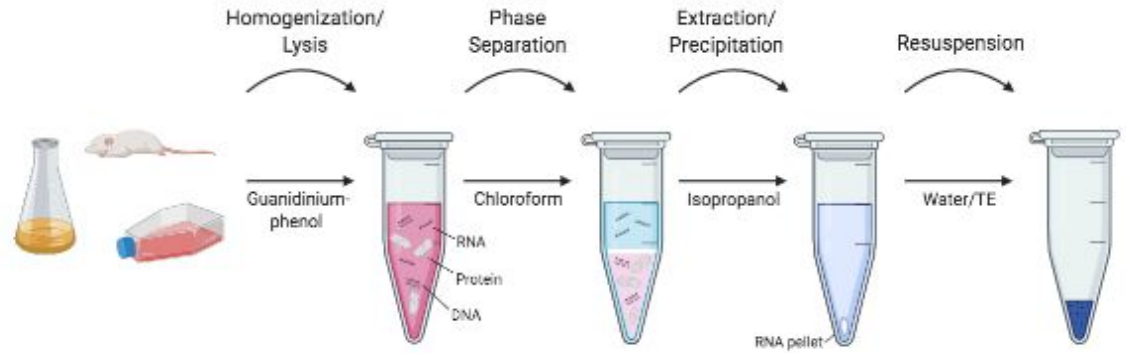
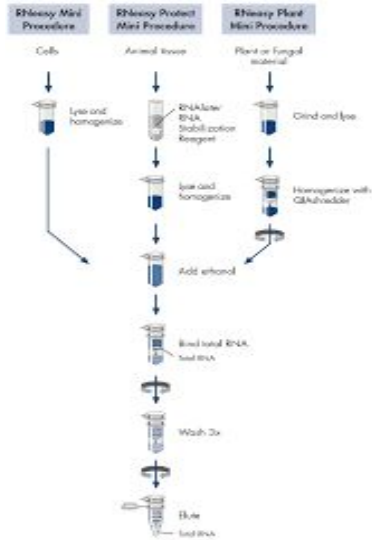
Sampling design

VERY IMPORTANT: What is your research question? Will you have enough to address your questions?

Things to bear in mind while carrying out RNA-Seq:

- 1) Is bulk RNA-Seq necessary ?
- 2) Conditions and Phenotypes
- 3) Replicates – accounts for variation and important to validate results
- 4) Consult sequencing specialists – for advice on sampling
- 5) # of replicants per condition/phenotype
- 6) RNA isolation protocol
- 7) RNA library prep (DIY or outsource?)
- 8) Analysis plan

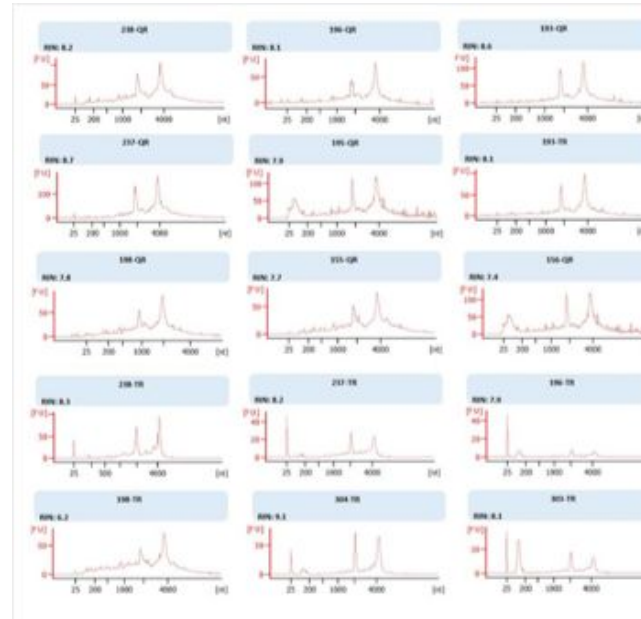
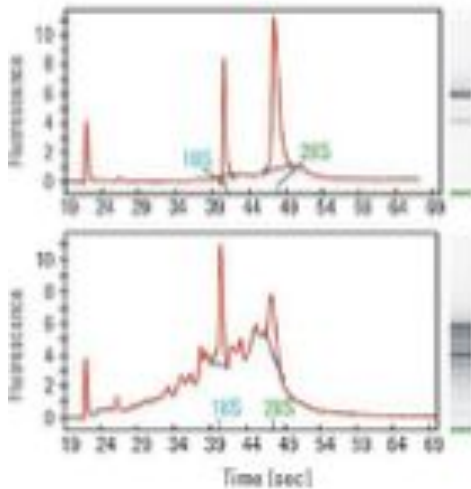
Obtaining the mRNA



RNA QC and quantification

It is important to establish both the purity and concentration of RNA that has been extracted

Agilent® 2100 Bioanalyzer



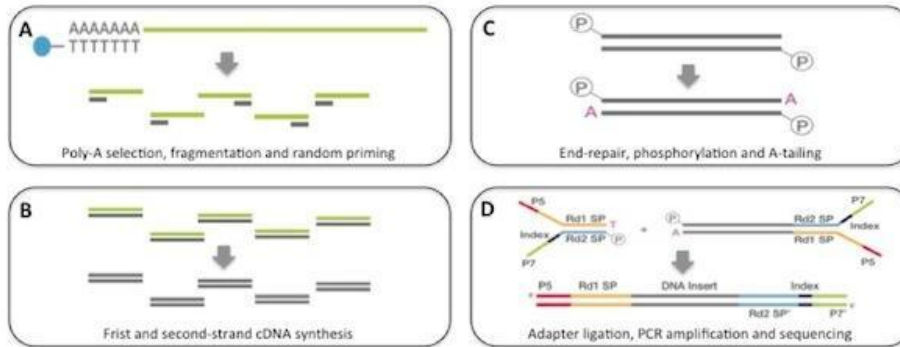
Bala *et al.*, 2016

RNA Sequencing

Whole transcriptome shotgun sequencing (WTSS)

- Reveals the presence and quantify of RNA in a biological sample at a given moment in /me

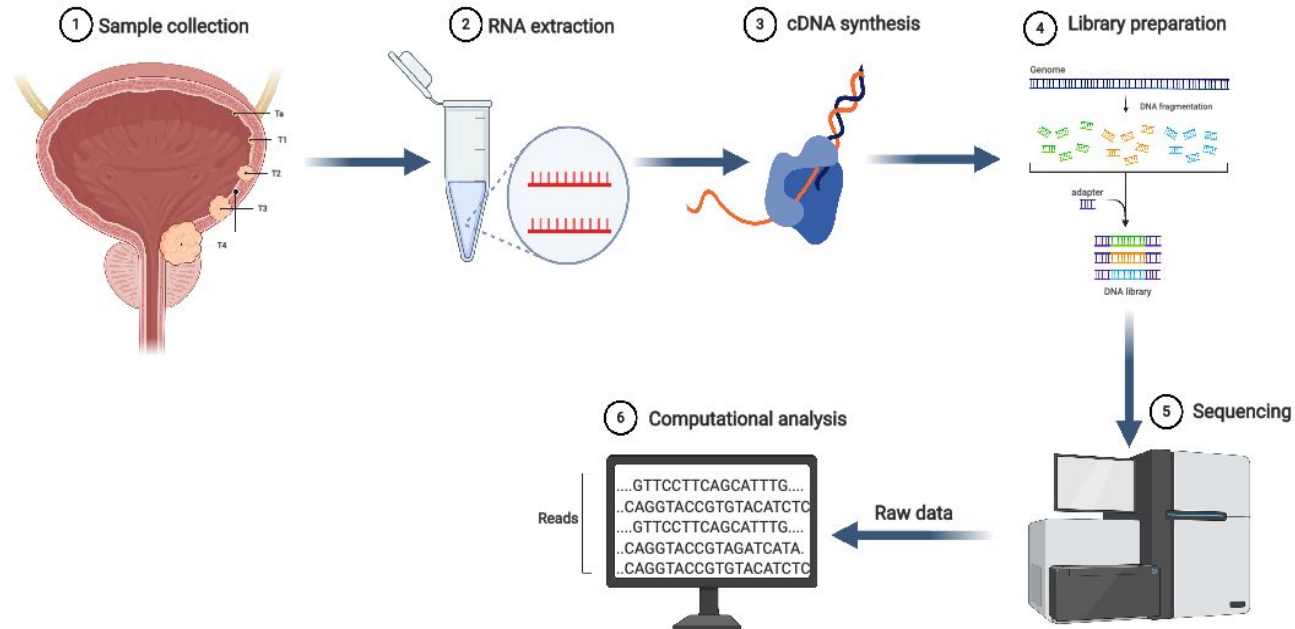
Illumina Tru-Seq RNA-seq protocol



Library prep begins from 100ng-1ug of Total RNA which is poly-A selected (A) with magnetic beads. Double-stranded cDNA (B) is phosphorylated and A-tailed (C) ready for adapter ligation. The library is PCR amplified (D) ready for clustering and sequencing.

Transcriptomics workflow

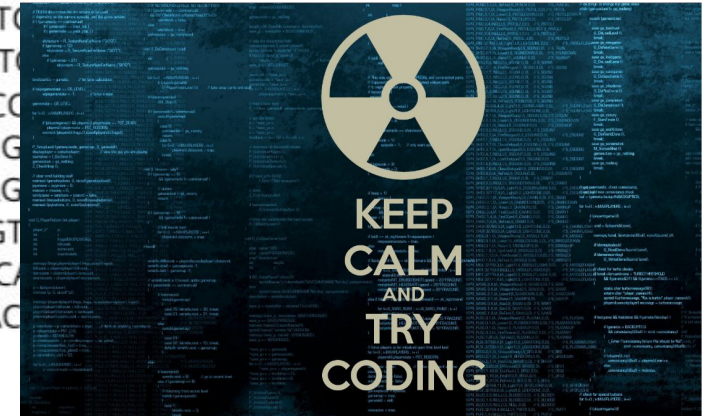
RNA sequencing workflow



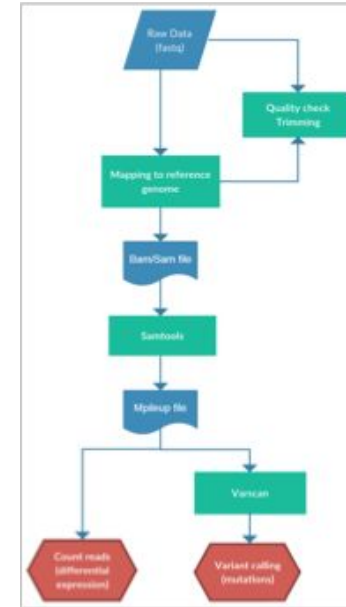
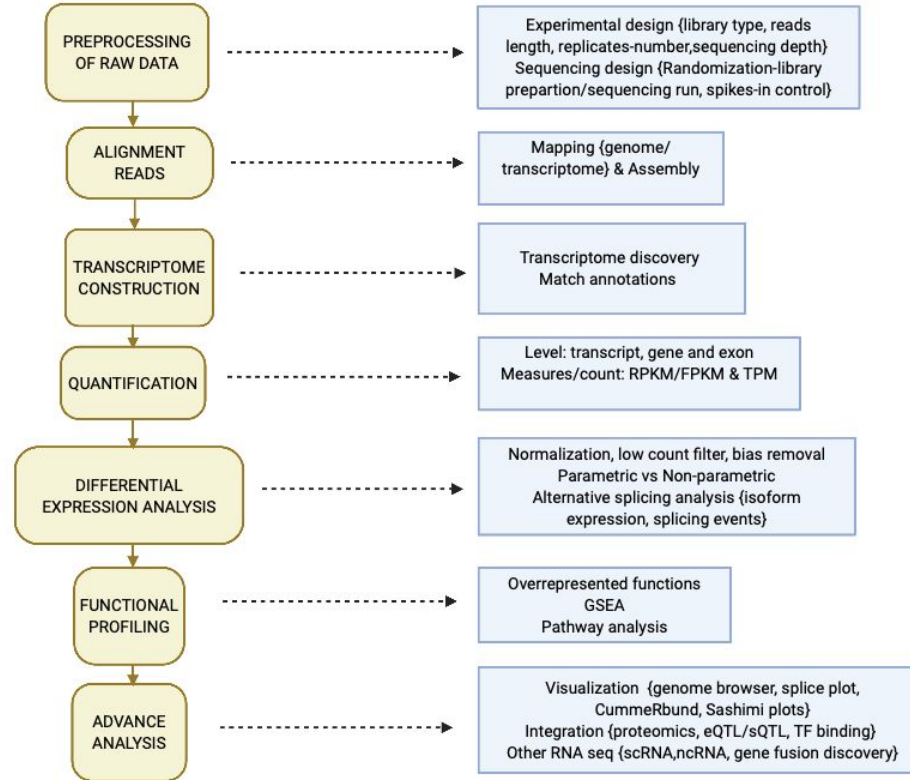
Bioinformatics – Analysis of transcriptomics data



```
umarahmad@umarahmad-VirtualBox:~$ ls
Desktop  Downloads  Pictures  Share     Videos
Documents Music      Public   Templates
umarahmad@umarahmad-VirtualBox:~$ cd Share/
umarahmad@umarahmad-VirtualBox:~/Share$ ls
BAB40370.1      lockdown-learning.Rproj  Q9BYF1.txt  testfile
BAB40370.1.fasta  'newfile '              temp        Umar.txt
BAB40370.1.txt   Q9BYF1.fasta            test        week1
umarahmad@umarahmad-VirtualBox:~/Share$
```



Transcriptomics pipeline



FASTQ files

Line 1: Sequence identifier

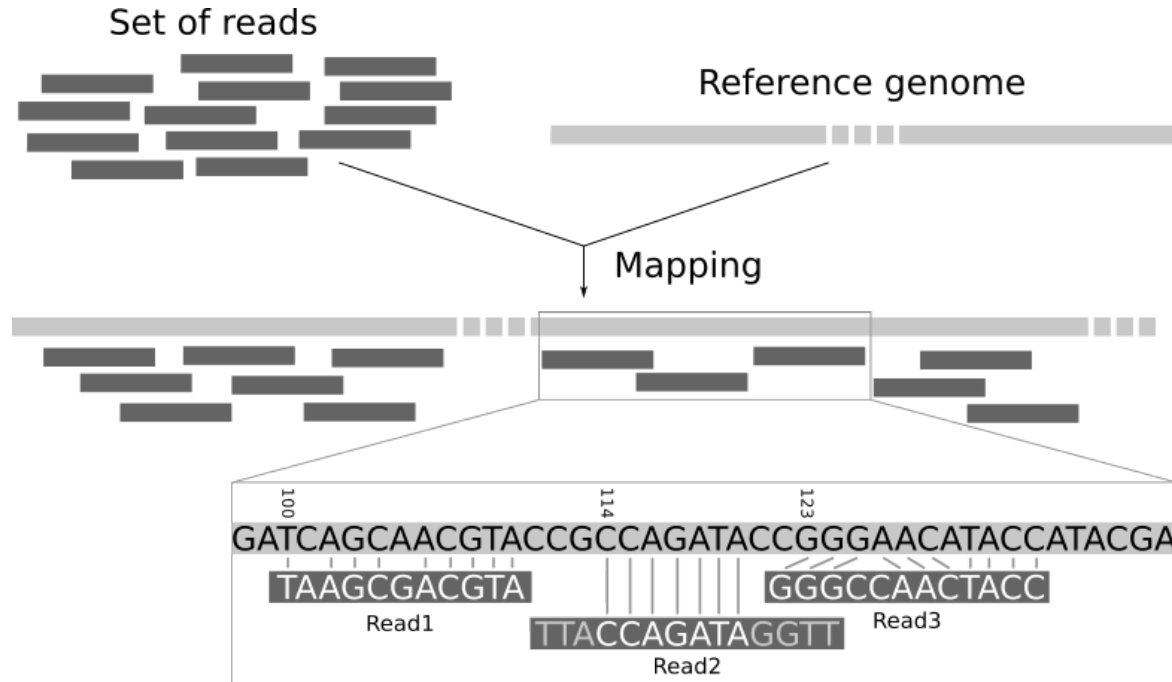
Line2: Raw sequence

Line3: meaningless

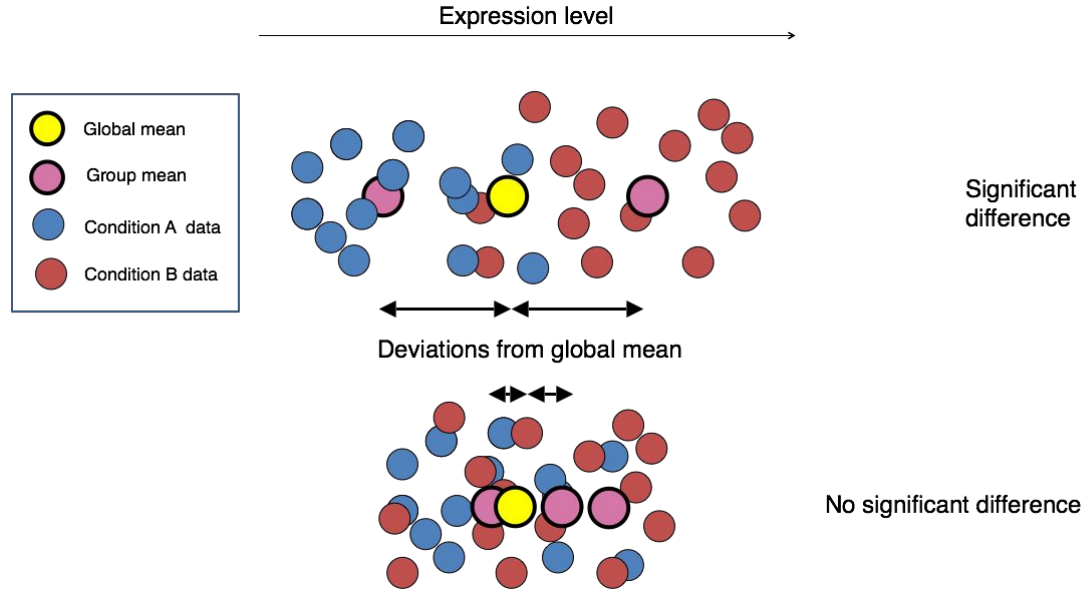
Line4: quality values for the sequence

[illegible]

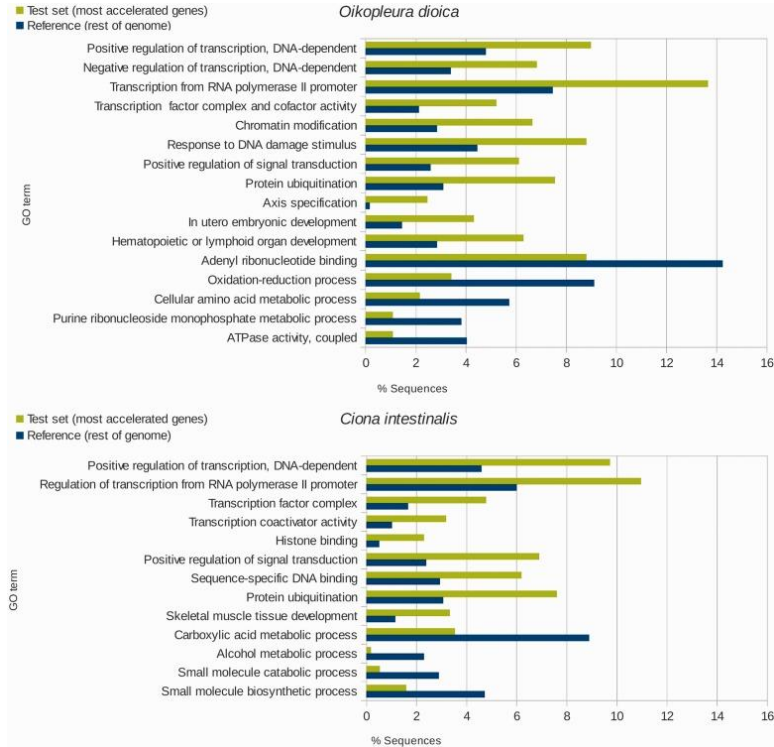
Align NGS reads to a reference genome



Analysis of Differentially Expressed Genes (DEGs)



Gene Ontology (GO) enrichment analysis

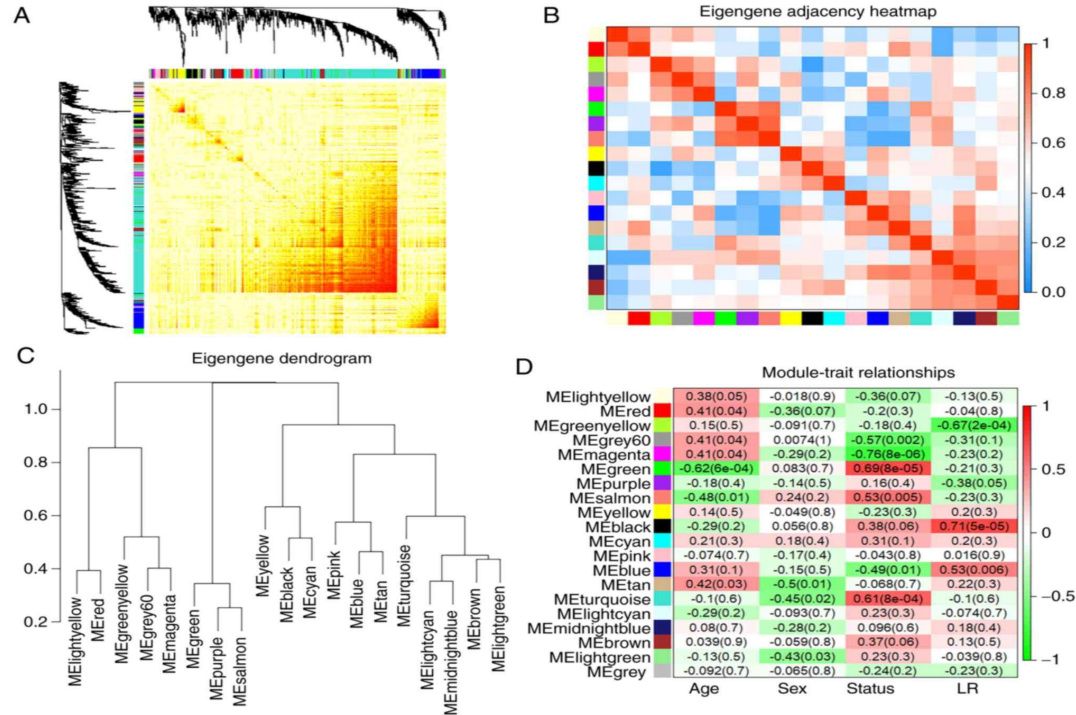


	Differential Expression	NO Differential Expression	Total
IN Transcription Elongation	12	3	15
NOT IN Transcription Elongation	3	12	15
Total	15	15	30

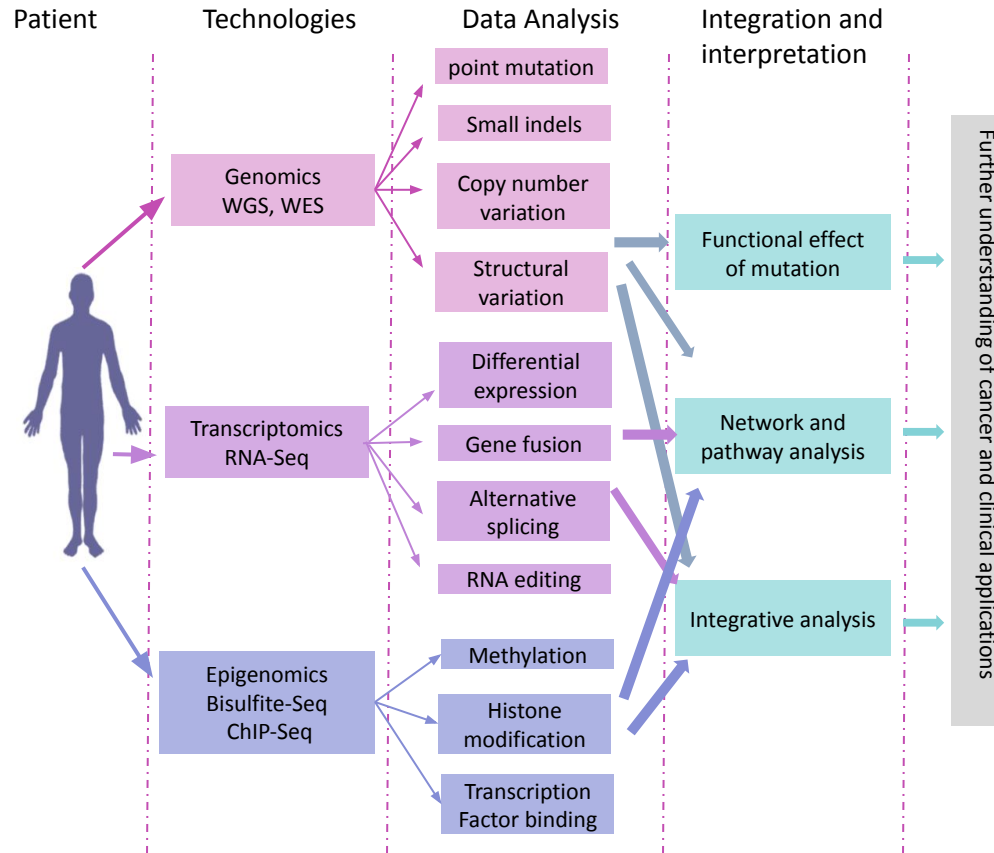
https://www.pathwaycommons.org/guide/primers/statistics/fishers_exact_test/

Figure 5 from: Berná, L, Alvarez-Valin, F. (2014). Evolutionary genomics of fast evolving tunicates. *Genome Biology and Evolution*. 6(7): 1724-1738

Co-expression network analysis



Application



Benefits and Challenges

Benefits:

- Independence on prior knowledge
- High resolution, sensitivity and large dynamic range
- Unravel previously inaccessible complexities

Challenge:

- Interpretation is not straightforward
- Procedures continue to evolve

Need help in your NGS analysis?

Consults:



Acknowledgments

Collaborative
members:

