Single cell transcriptomics (2):

Complex design, trajectory inference, somatic mutations

BBMS 3009: Genome Science (First Semester, 2021)

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Today’s learning objectives

1. Complex experiment designs and batch effects
2. Differentiation trajectory inference
3. Splicing and RNA velocity
4. Somatic mutations and its impact on gene expression

Resources:

- Analysis of single cell RNA-seq data (Sanger course) https://www.singlecellcourse.org/
- HKUMed Single-cell analysis tutorial workshop (HKU Med) https://statbiomed.github.io/HKU-single-cell-workshop/

Complex experiment designs

- Identify key abnormal cell types in Multiple sclerosis vs healthy donors
  - Design: 30 MS patient + 30 healthy donors, each with 2K cells

- Understand the cell type specific impact of a certain treatment
  - Design: 5 treated + 5 control, each with 5K cells

- Understand the genetic effects on cell differentiations
  - iPS cells differentiating to neurons, with 5 time points, 3K cells each time
  - For genetic, repeating for 100 cell lines

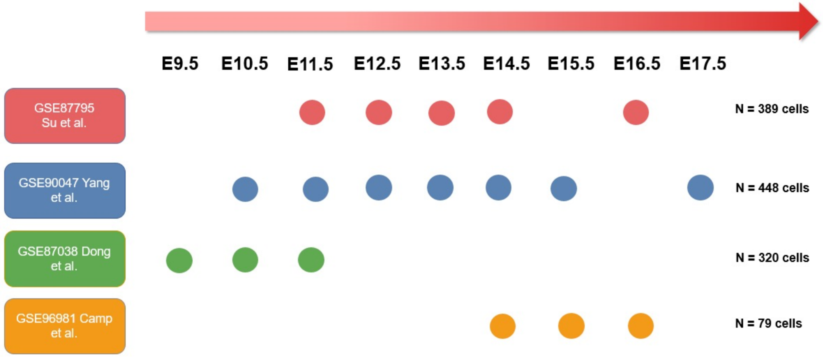
Multiple samples are needed: multiple batches are often needed;
Be careful what “sample” means: individuals or cells

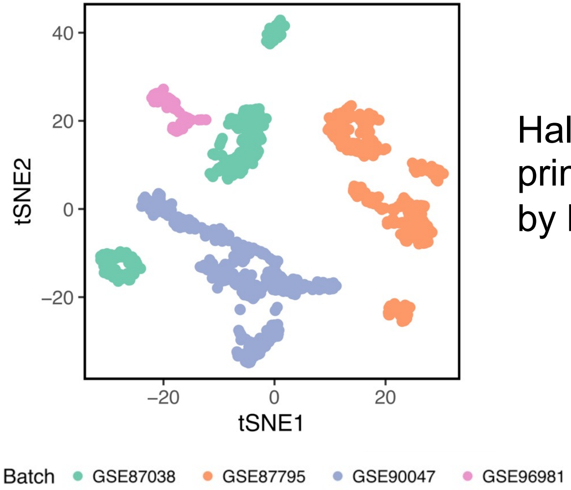
Batch effects

Batch 1

Batch 2

Batch effect: differences in gene expression caused by non-biological factors that may cause systematic difference in data generated in different batches of experiments

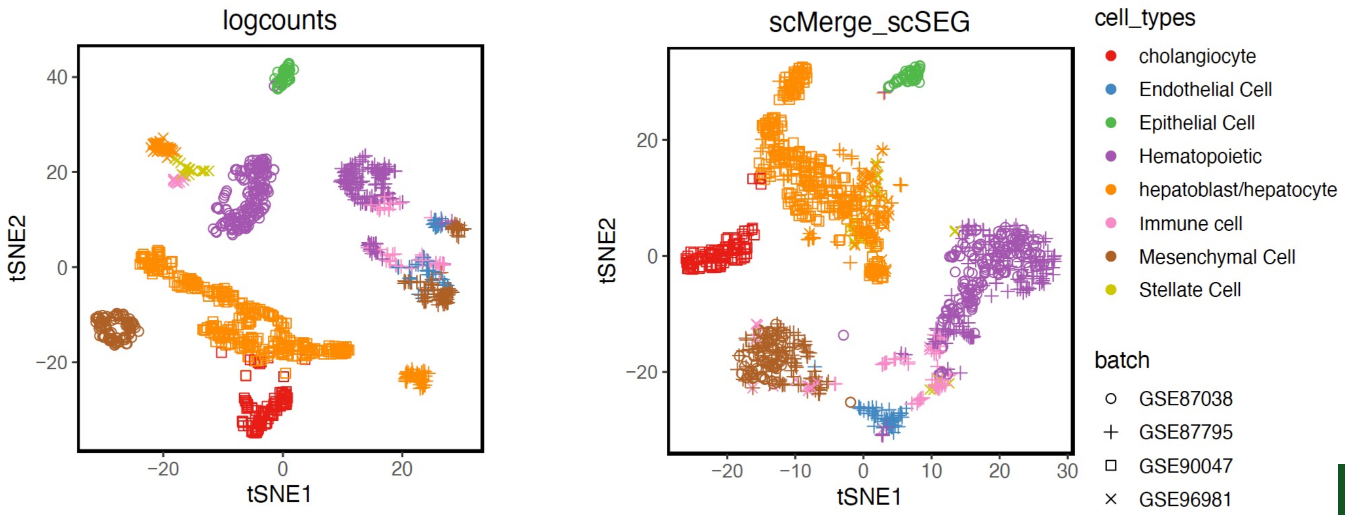




Hallmark: Cells are primarily grouped by batch

Batch effect correction in a post-step

- Preprocess each dataset separately (e.g., PCA), then align multiple datasets with statistical methods
- Many methods have been proposed to correct batch effects, e.g., scMerge
  - See a benchmark study: Tran et al, Gen Biol, 2020.
- Trade-off: under correction vs over correction.
  - Aim to regress out technical batch effects but retain biological differences



Lin et al (2019) PNAS

Less batch effect with join analysis

- Another approach is to join analyzing all multiple datasets, e.g., align them to a common (reference, often large) dataset
  - Conventional statistical methods: project all datasets into a common PCA space; Liu et al, Nat Biotech, 2021
  - Machine learning methods: transfer learning, e.g., Lotfollahi et al, nat biotech, 2021; methods not so mature, but shows good potential

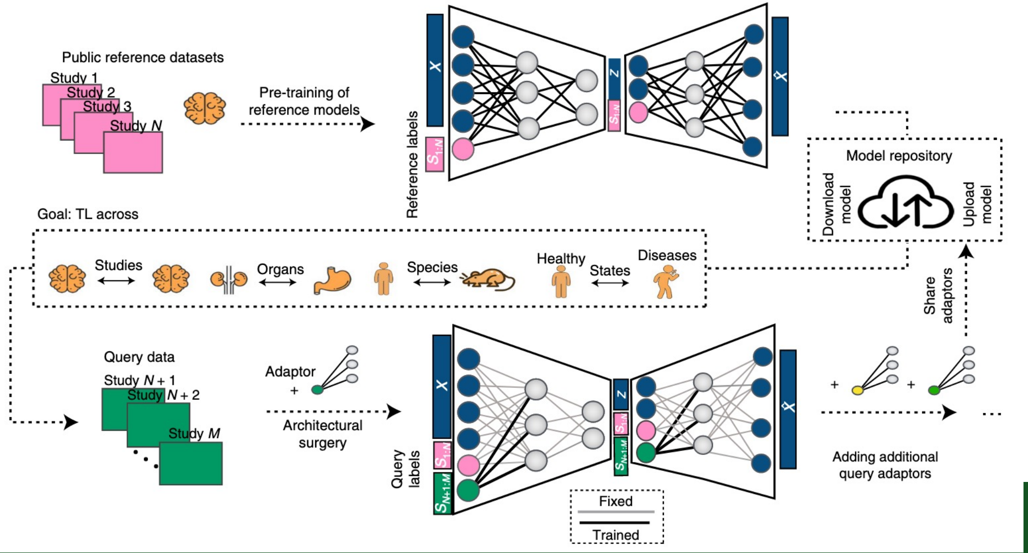
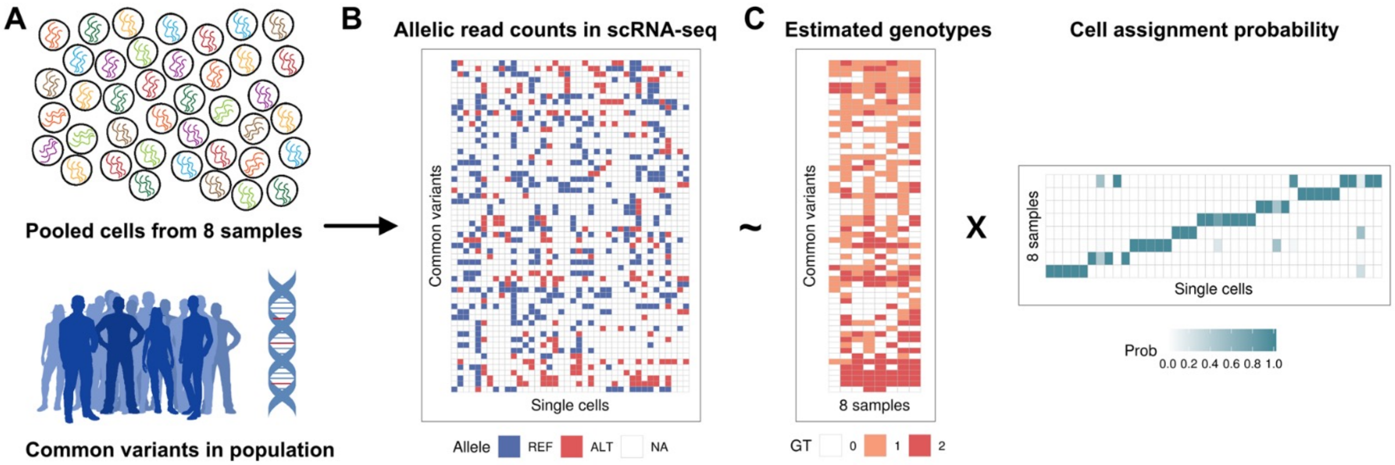


Illustration of a transfer learning method scArches

Multiplexed settings

- Given the cells are barcoded, multiple samples can be further multiplexed by using natural genetic makeups, or external molecular barcodes
- Batch effect in the sequencing step can be eliminated



Huang et al, 2019. Vireo: genetic makeups without reference;
Kang et al. 2018. Demuxlet: genetic makeup;
Shin et al, 2019. BSO: molecular barcoding.

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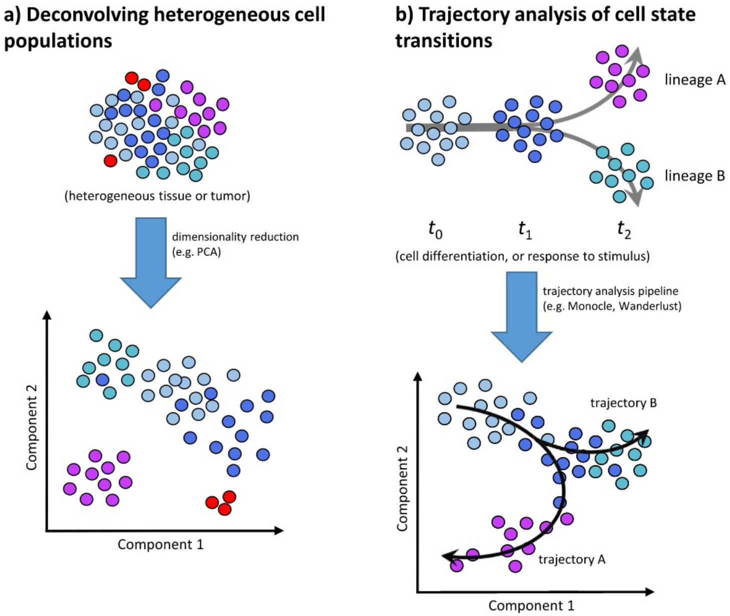
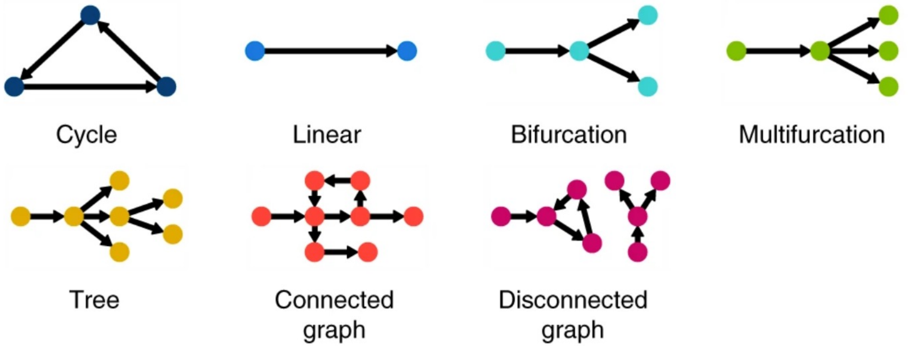
Trajectory inference

- The pseudo-temporal ordering of the cells in development or differentiation


a) Deconvolving heterogeneous cell populations
(heterogeneous tissue or tumor)
dimensionality reduction (e.g. PCA)
Component 2
Component 1
b) Trajectory analysis of cell state transitions
lineage A
lineage B
t_0
t_1
t_2
(cell differentiation, or response to stimulus)
trajectory analysis pipeline (e.g. Monocle, Wanderlust)
trajectory B
trajectory A
Component 2
Component 1

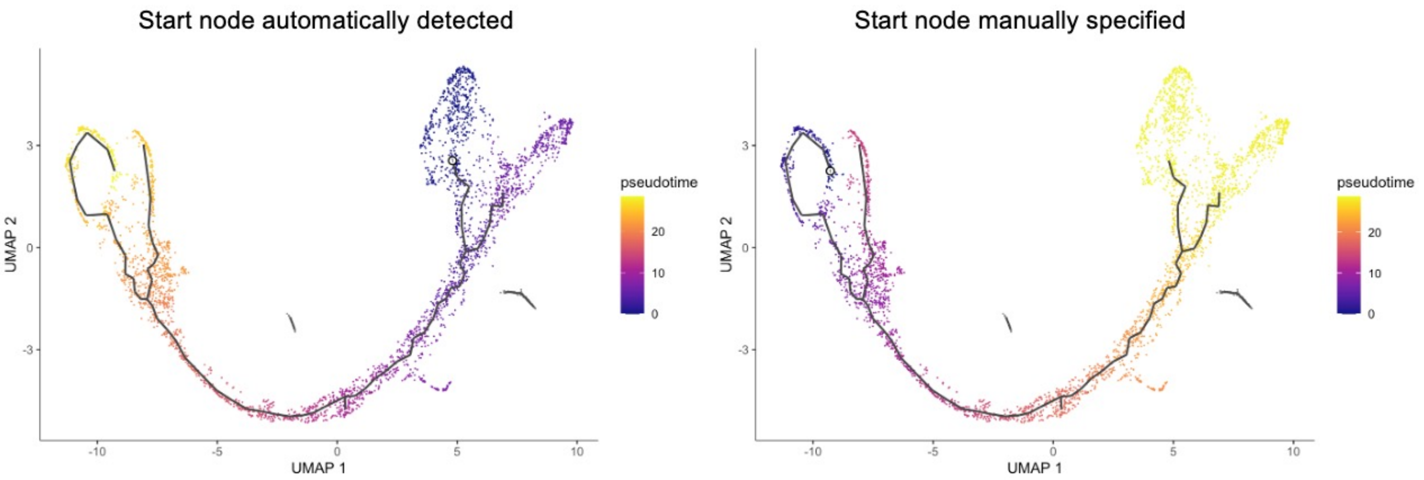


An inferred trajectory may or may not represent real developmental lineage (which required genetic linage tracing experiment)

Liu and Trapnell, F1000, 2019; Saelens et al. Nat biotech, 2019

Trajectory inference: directionality

- Commonly based on the Euclidean distance of transcriptome between two cells in raw genes or PCA space.
- Intrinsic challenge: very weak information on directionality along an inferred trajectory. No info for past and future states.



Start node automatically detected

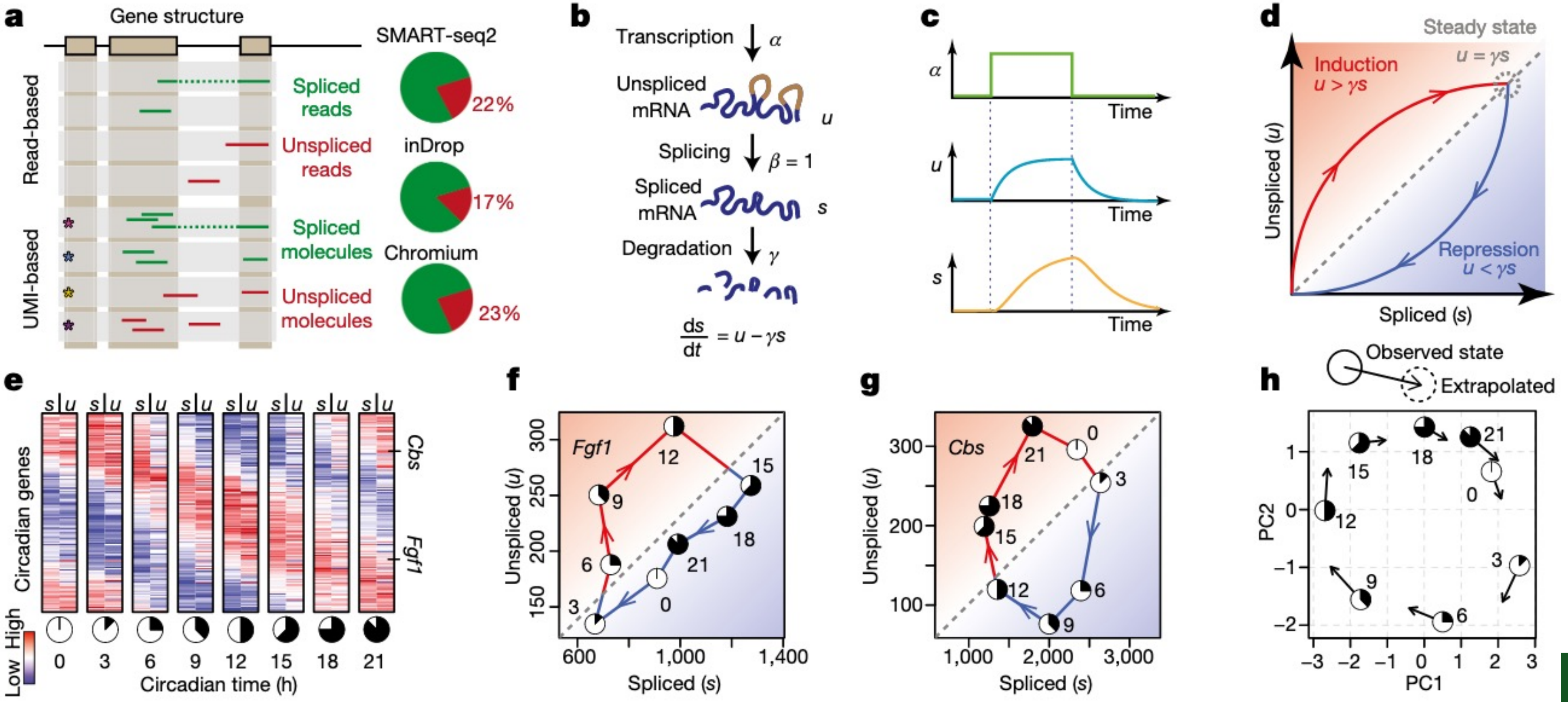
Start node manually specified

Monocl3: Pseudo-time requires specifying starting node

La Manno et al, Nature, 2018

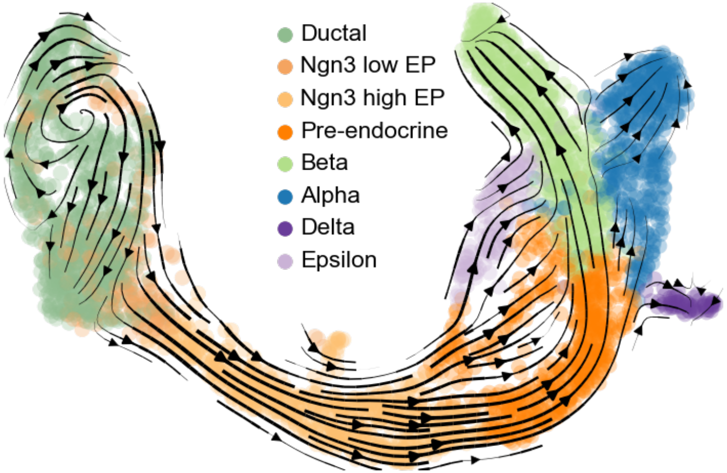
RNA velocity offers directionality

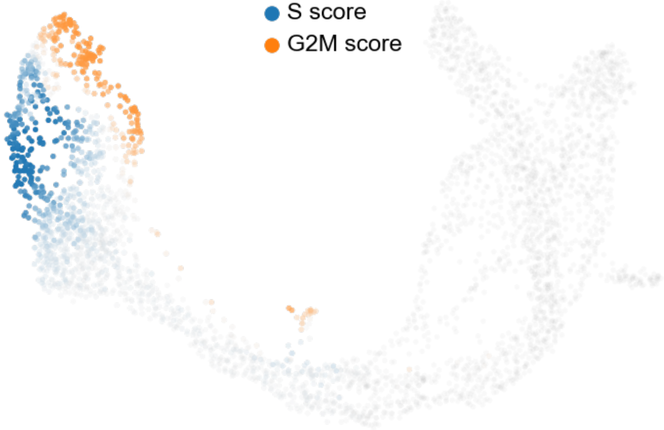
- Intrinsic dynamics of RNA processing (single direction)
- The level of unspliced RNAs indicate the future level of spliced RNAs



RNA velocity: projection of transitions

- Using unspliced RNAs to predict the near future of each gene
- Find the most similar transcriptome to the predicted one from unspliced RNAs, as the predicted cell state in near future



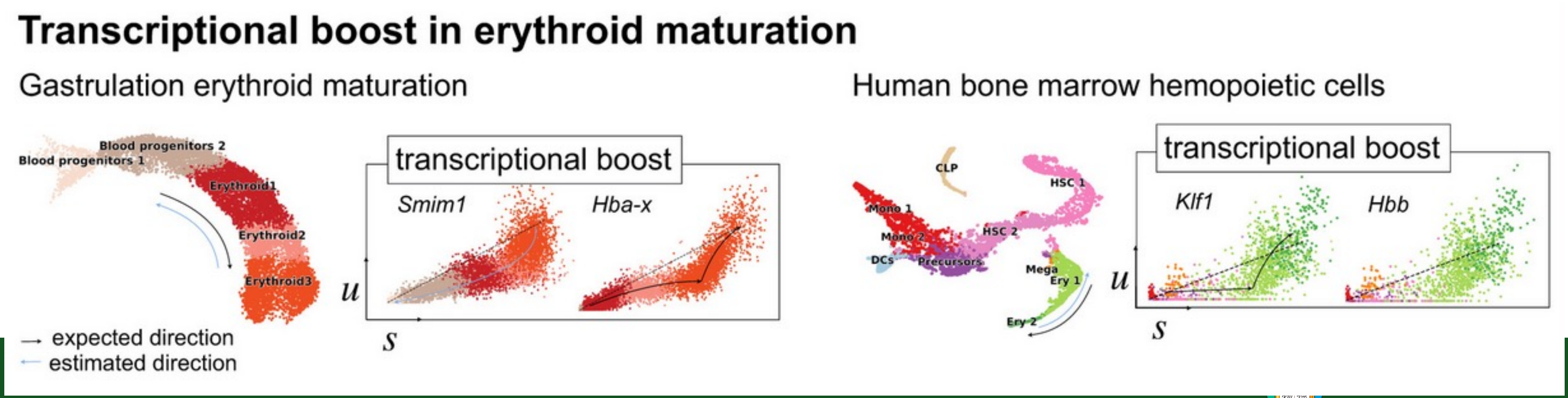


Bergen et al, Nat Biotech, 2020; scvelo, a Python package for RNA velocity analysis

Bergen, et al. MSB, 2021

RNA velocity: potential limitations

- Major limitations to be addressed, despite the appealing concept
  - Multiple kinetic rates (splicing rates, degradation rates) across populations
  - Transcriptional boost (far from steady state)
  - Complex kinetics (multiple branching)
- Potential ideas to improve the robustness and accuracy of RNA velocity:
  - Select informative genes: BRIE2 (Huang & Sanguinetti, 2021)
  - Denoise by projection on lower dimensions: veloAE (Qiao & Huang, 2021)



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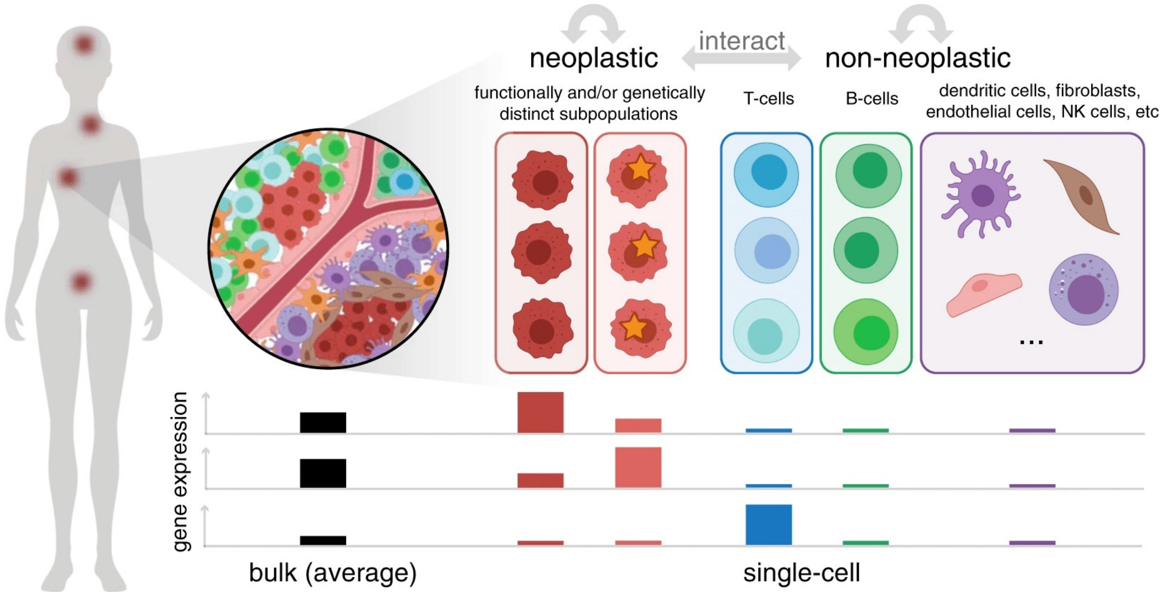
Cellular composition of a tumour

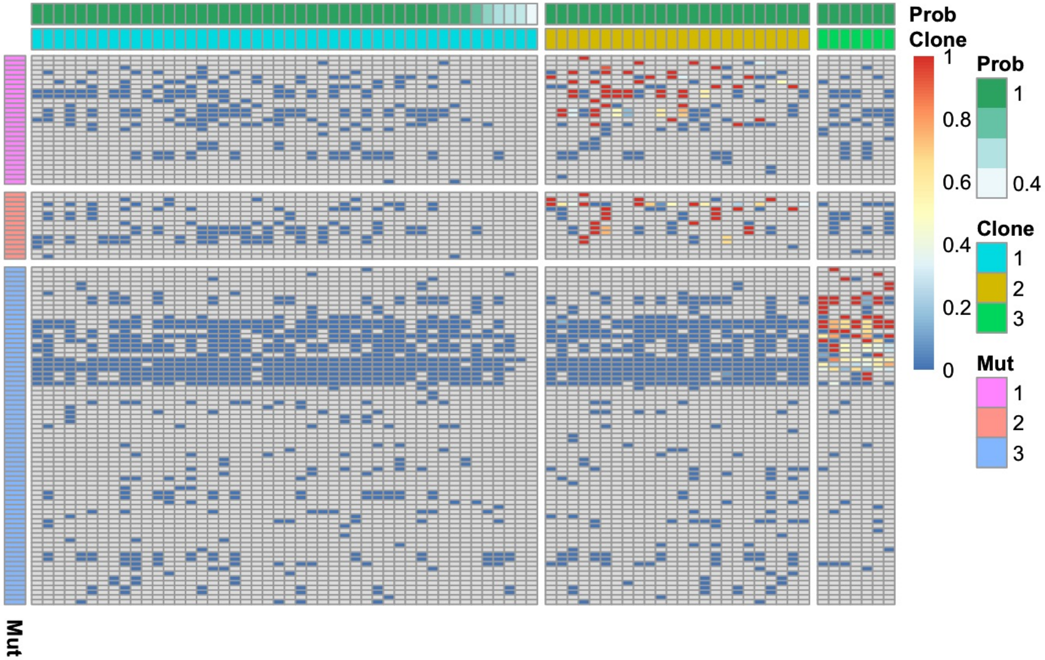

neoplastic
interact
non-neoplastic
functionally and/or genetically distinct subpopulations
T-cells
B-cells
dendritic cells, fibroblasts, endothelial cells, NK cells, etc
...
gene expression
bulk (average)
single-cell

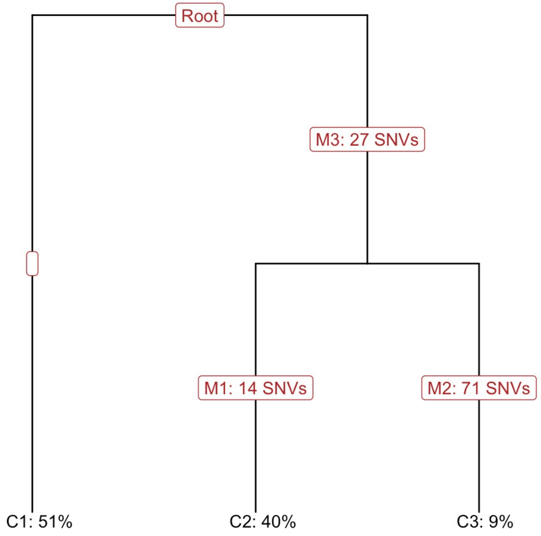
Neoplastic cells (cells with abnormal growth), usually affected by genetic alteration

Non-neoplastic cells (tumour microenvironment):
- Tumour infiltrating lymphocytes (TILs), including T cells, B cells, macrophages, etc
- Stromal cells, including cancer-associated fibroblast (CAFs)

Single-nucleotide variant (SNV)

- SNVs can be observed in the expressed RNAs
- Grouping cells by their carrying SNVs → clonal SNVs

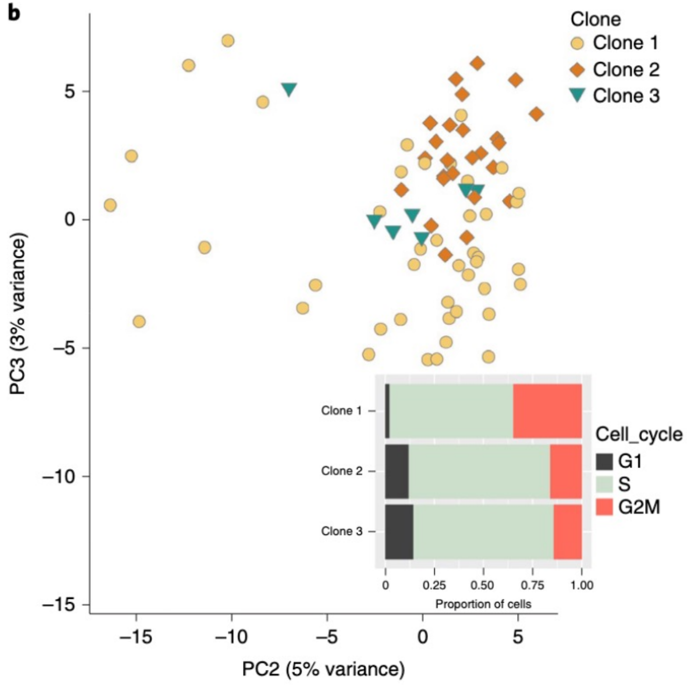


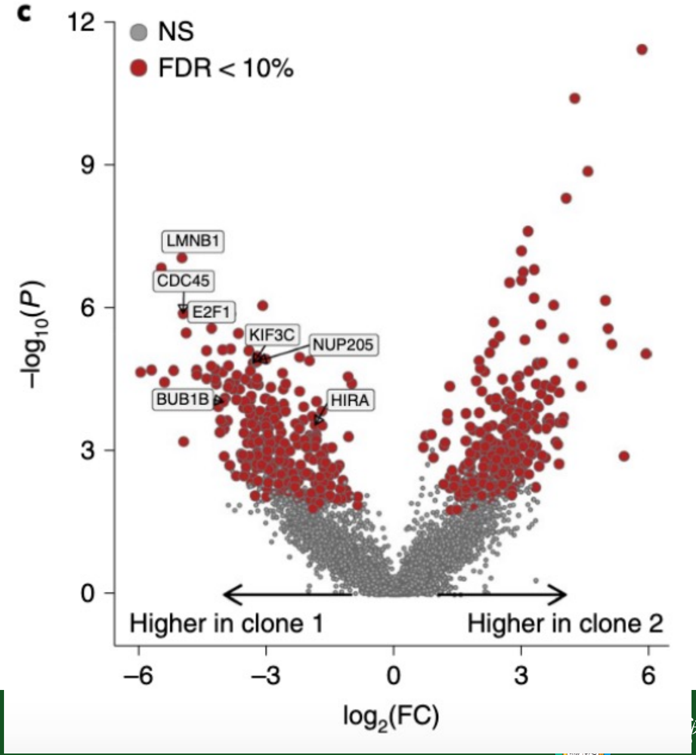


McCarthy, Rostom, Huang, et al, Nat Meth, 2020

Clonal SNVs and impact on gene expression

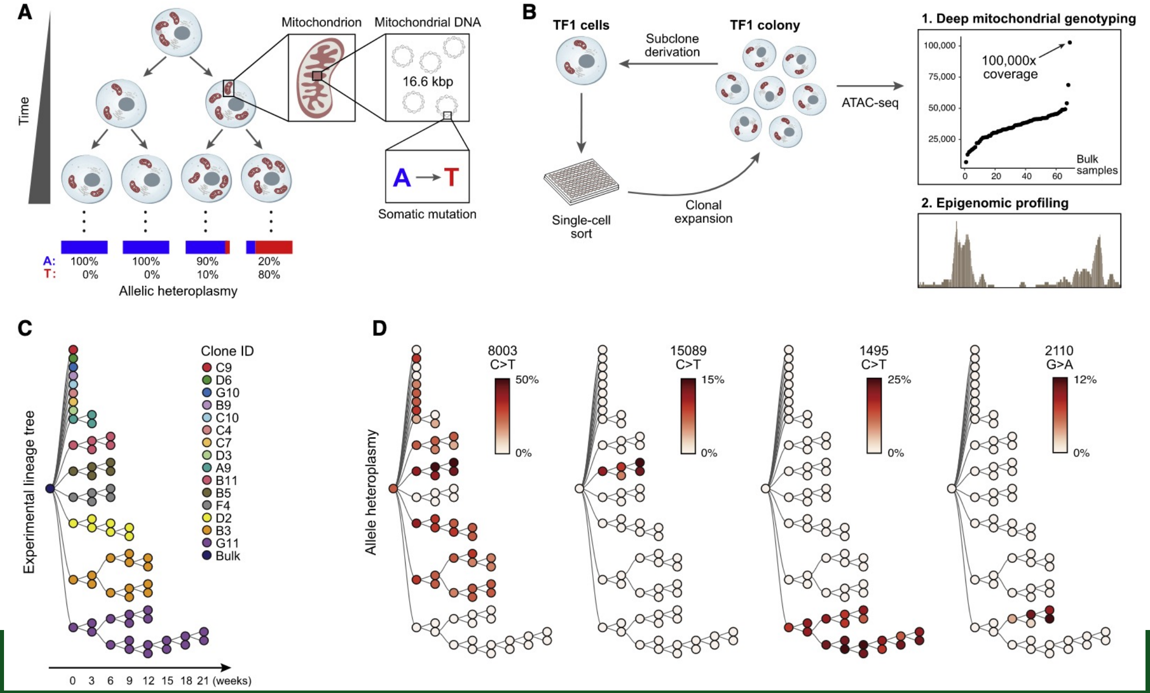
- Global impact on transcriptome; separation on PCA space
- Detecting differentially expressed genes between clones (recall how?)





Mitochondrial mutations

- Challenges in SNV: coverage is low (SMART-seq) or very low (droplet)
- mtDNA variants: much higher coverage (many copies)
- High mutation rate: potentials for lineage tracing

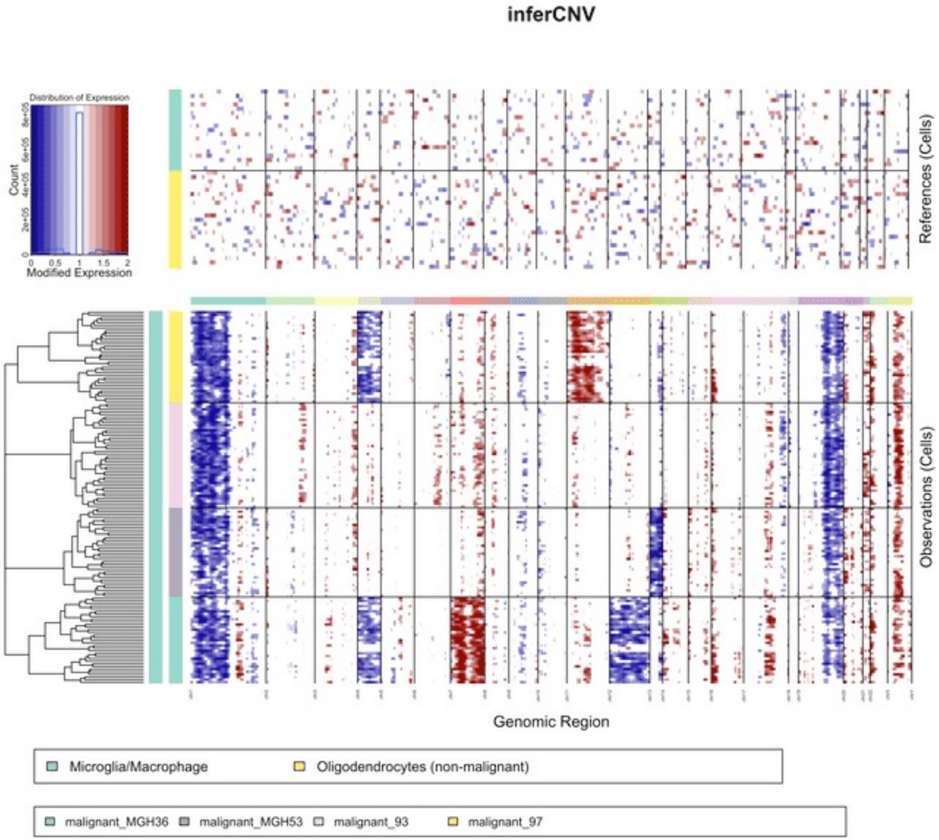


Accurate detection clonal mtDNA variants:

- mgatk: Lareau et al, 2020;
- MQuad: Kwok et al, 2021

Ludwig et al, Cell, 2019

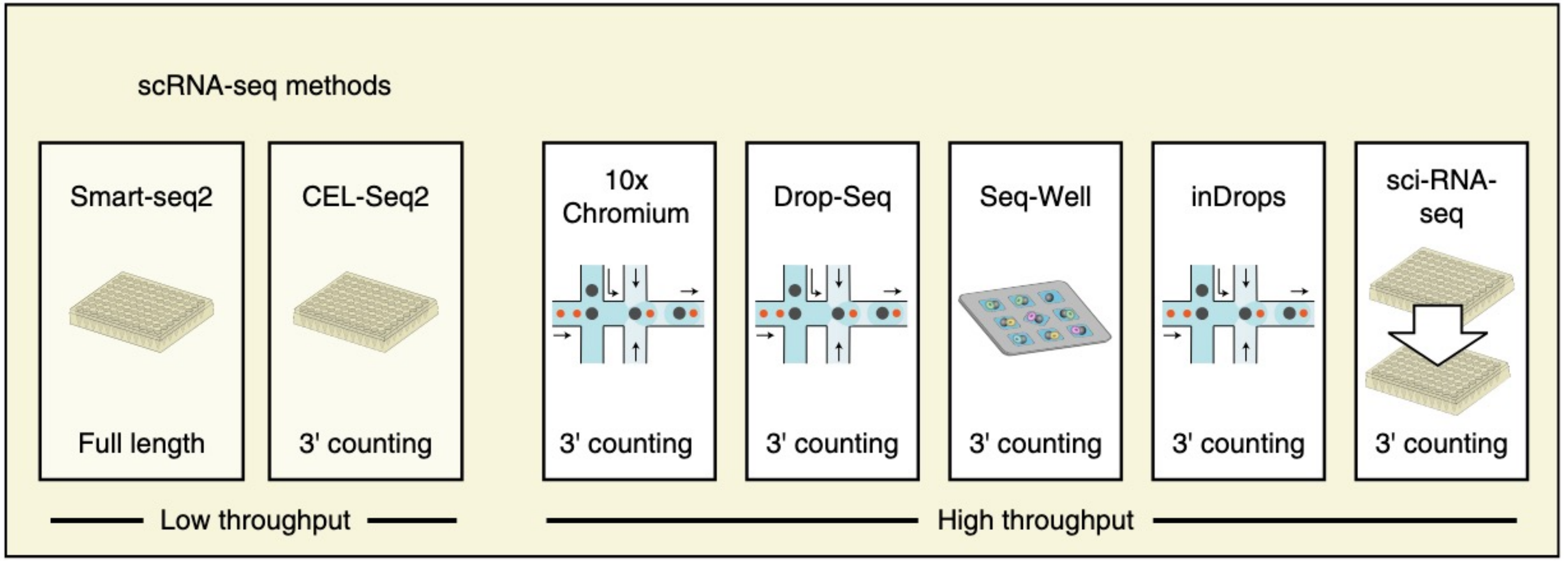
Copy number variation



- CNVs commonly exist in tumour cells
- CNV clonal structure
- Large range of CNV, whole chromosome or an arm
- Commonly used methods: inferCNV, CopyKat

- **Open challenges**:
  - Detecting loss of heterozygosity (allelic info)
  - Integrating with other assays, bulk WGS

Choice of protocols for somatic mutations



	Example protocol	coverage	cell numbers	missing regions	cost	CNV	SNV	mtSNV
scDNA-seq (well-based)		moderate	low	moderate	high	Very good	Good	Good
scDNA-seq (droplet)	10x CNV	low	moderate	high	high	Very good	Poor	Moderate
scDNA-seq (targeted)	Mission Bio	high (target)	high	non-targeted	moderate	Maybe	Good	Maybe
scRNA-seq (well-based)	SMART-seq2	moderate	low	non-expressed	moderate	Good	Moderate	Good
scRNA-seq (droplet)	10x Genomics	low	high	non 3' or 5'	moderate	Good	Very poor	Poor
scRNA-seq (targeted)	GoT	high (target)	high	non-targeted	moderate	Maybe	Good	Poor

Public resources for single-cell data

- Human Cell Atlas
  https://www.humancellatlas.org/

- Mouse Organogenesis Cell Atlas
  https://oncoscape.v3.sttrcancer.org/atlas.gs.washington.edu.mouse.rna/landing

- Tabula Muris Senis
  https://tabula-muris-senis.ds.czbiohub.org/

- EBI Single Cell Expression Atlas:
  https://www.ebi.ac.uk/gxa/sc/

Any questions?

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Contents and assessment level

- Quantitative skills for genomic data analysis 1
  - Only for assignment
- Quantitative skills for genomic data analysis 2
- Transcriptomics 1
- Transcriptomics 2
  - Multiple gene analysis: Only for assignment
- Expression QTL 1
- Expression QTL 2
  - Machine learning, gene regulatory network: only for introduction
- Single cell transcriptomics 1
  - Clustering and cell type annotation: only for introduction
- Single cell transcriptomics 2
  - Trajectory inference, RNA velocity: only for introduction

Introduction level means understand the concept

Focus:
Hypothesis testing and its application in DEG, eQTL, and highly variable genes

Drop-in session (Zoom or my office)

You can join either or both slots; no appointment is needed.

Slot 1: 30th Sep (Thursday), 3-4pm

Slot 2: 13th Oct (Friday), 2-3pm

Join from Zoom:

Topic: BBMS3009 open office hour - Dr. Huang

Join Zoom Meeting

https://hku.zoom.us/j/91480501144

Meeting ID: 914 8050 1144

Or come to my office:

My office: 1-05E, 1/F, JCBIR, 5 Sassoon Road