

Single Cell Biological Applications

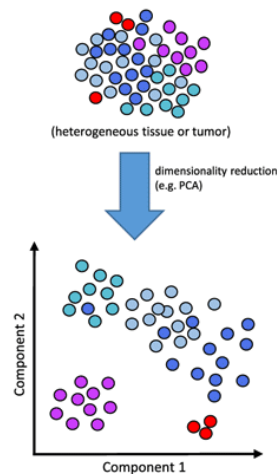
*Agnès Paquet
SincellTE, Roscoff 04/02/2019*



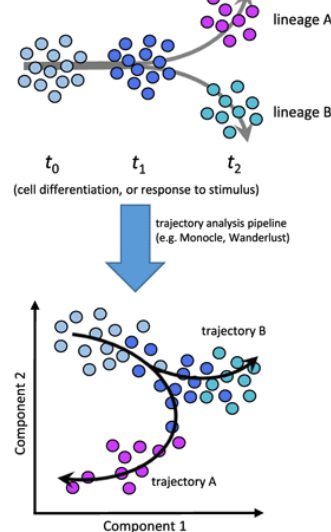
Wide range of applications

1. Resolving cellular heterogeneity
2. Understand developmental processes and cell fate decisions
3. Fundamental characteristics of gene expression
4. Identification of co-regulated gene modules and network inference

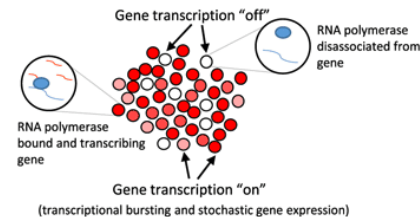
a) Deconvolving heterogeneous cell populations



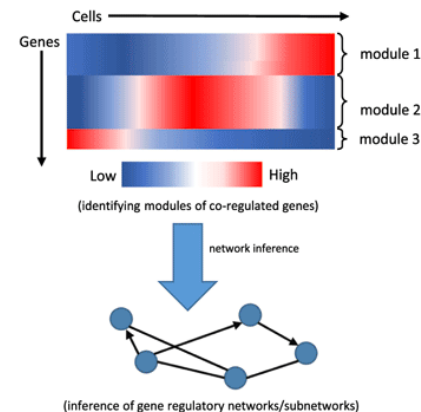
b) Trajectory analysis of cell state transitions



c) Dissecting transcription mechanics



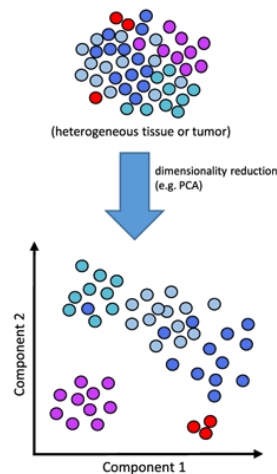
d) Network inference



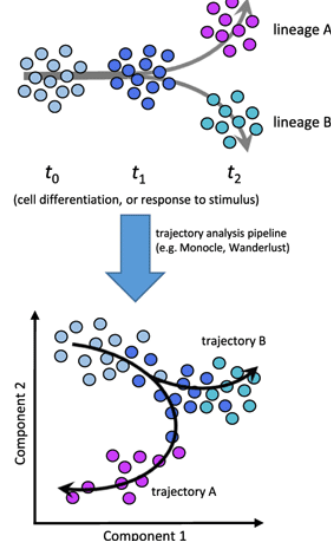
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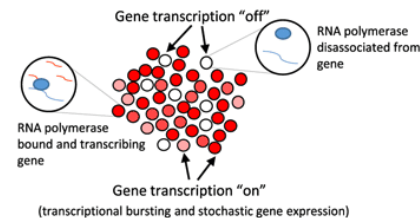
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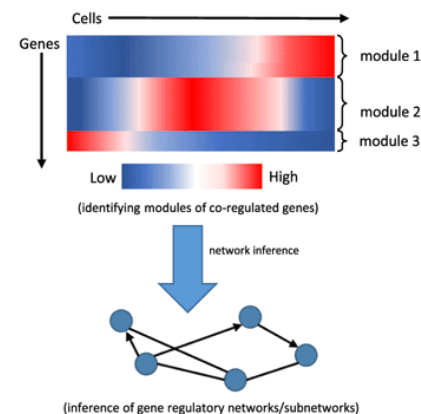
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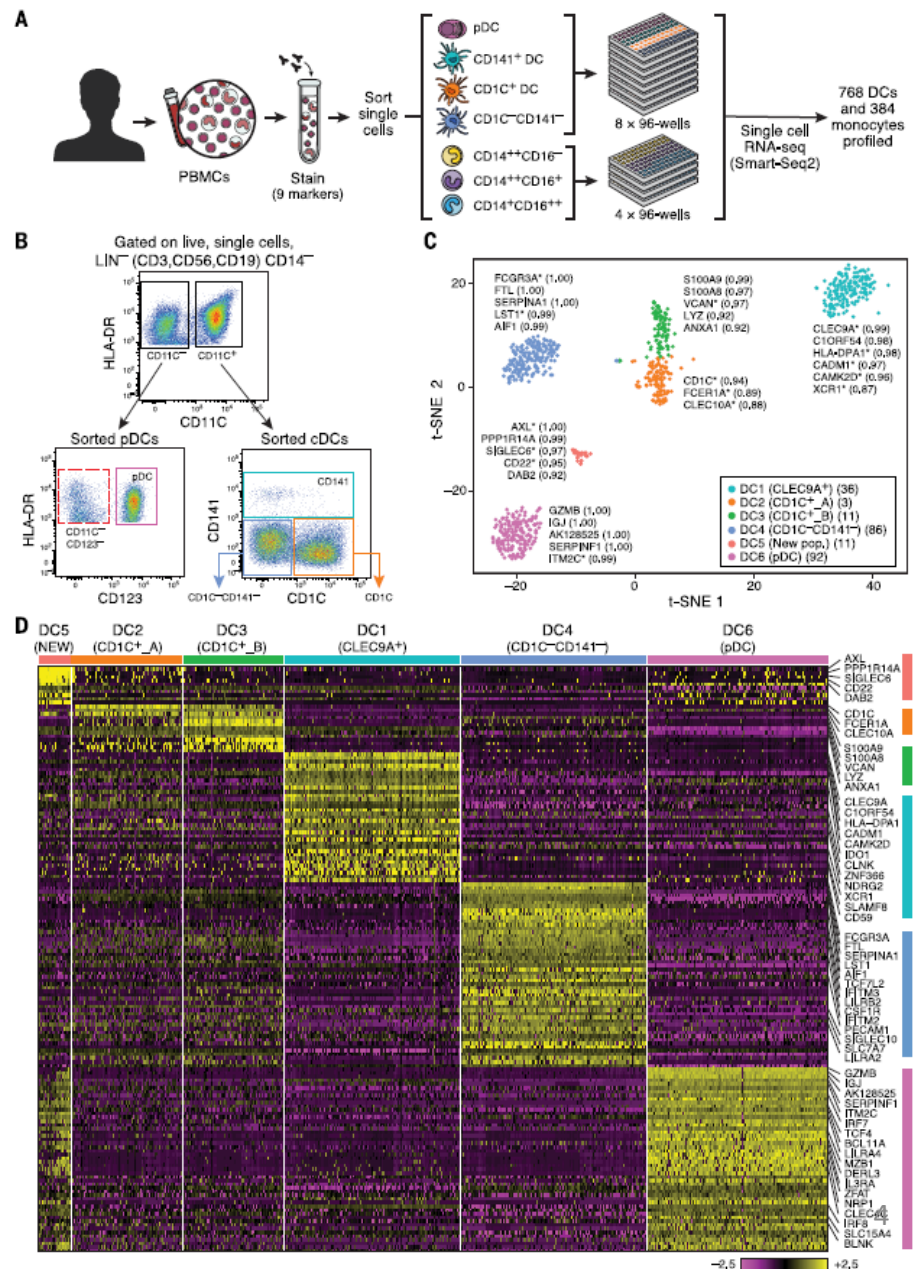


RESEARCH ARTICLE SUMMARY

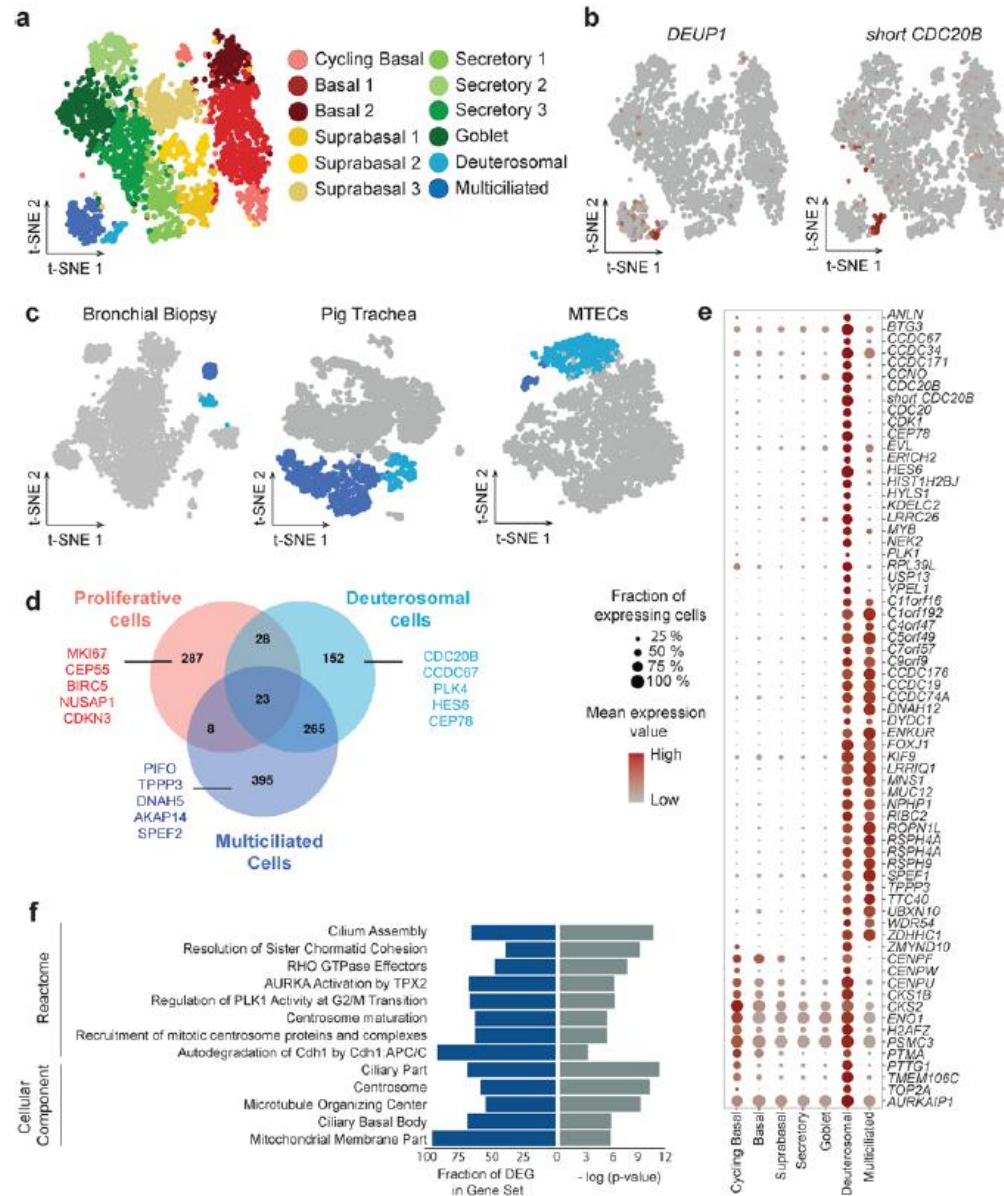
IMMUNOGENOMICS

Single-cell RNA-seq reveals new types of human blood dendritic cells, monocytes, and progenitors

Alexandra-Chloé Villani,*† Rahul Satija,* Gary Reynolds, Siranush Sarkizova, Karthik Shekhar, James Fletcher, Morgane Griesbeck, Andrew Butler, Shiwei Zheng, Suzan Lazo, Laura Jardine, David Dixon, Emily Stephenson, Emil Nilsson, Ida Grundberg, David McDonald, Andrew Filby, Weibo Li, Philip L. De Jager, Orit Rozenblatt-Rosen, Andrew A. Lane, Muzlifah Haniffa,† Aviv Regev,† Nir Hacohen†



New marker discovery



- Profiling of gene expression level changes during infection
- Study of virus-infected cells (HIV-1,...)
- Bioinformatics:
 - If virus genes are polyA, they can be profiled at the same time that host genes
 - Think about normalization strategy: viral RNA quantity is increasing during infection, host genes RNA is constant

eLife. 2018; 7: e32303.

Published online 2018 Feb 16. doi: [10.7554/eLife.32303](https://doi.org/10.7554/eLife.32303)

PM

Extreme heterogeneity of influenza virus infection in single cells

[Alistair B Russell](#)¹, [Cole Trapnell](#)² and [Jesse D Bloom](#)^{1,2}

Arup K Chakraborty, Reviewing Editor

Arup K Chakraborty, Massachusetts Institute of Technology, United States;

[Author information](#) ► [Article notes](#) ► [Copyright and License information](#) ► [Disclaimer](#)

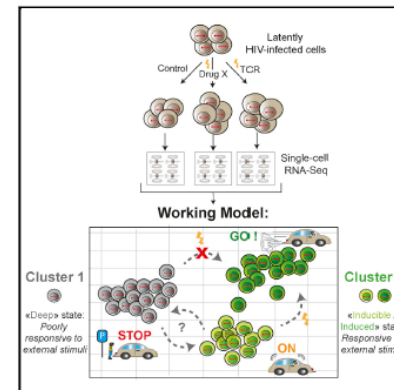
See "Single-cell transcriptional dynamics of flavivirus infection" in volume 7, e32942.

This article has been [cited by](#) other articles in PMC.

Cell Reports

Single-Cell RNA-Seq Reveals Transcriptional Heterogeneity in Latent and Reactivated HIV-Infected Cells

Graphical Abstract



Authors

Monica Golumbeanu, Sara Cristinelli, Sylvie Rato, Miguel Munoz, Matthias Cavassini, Niko Beerenwinkel, Angela Cluffi

Correspondence

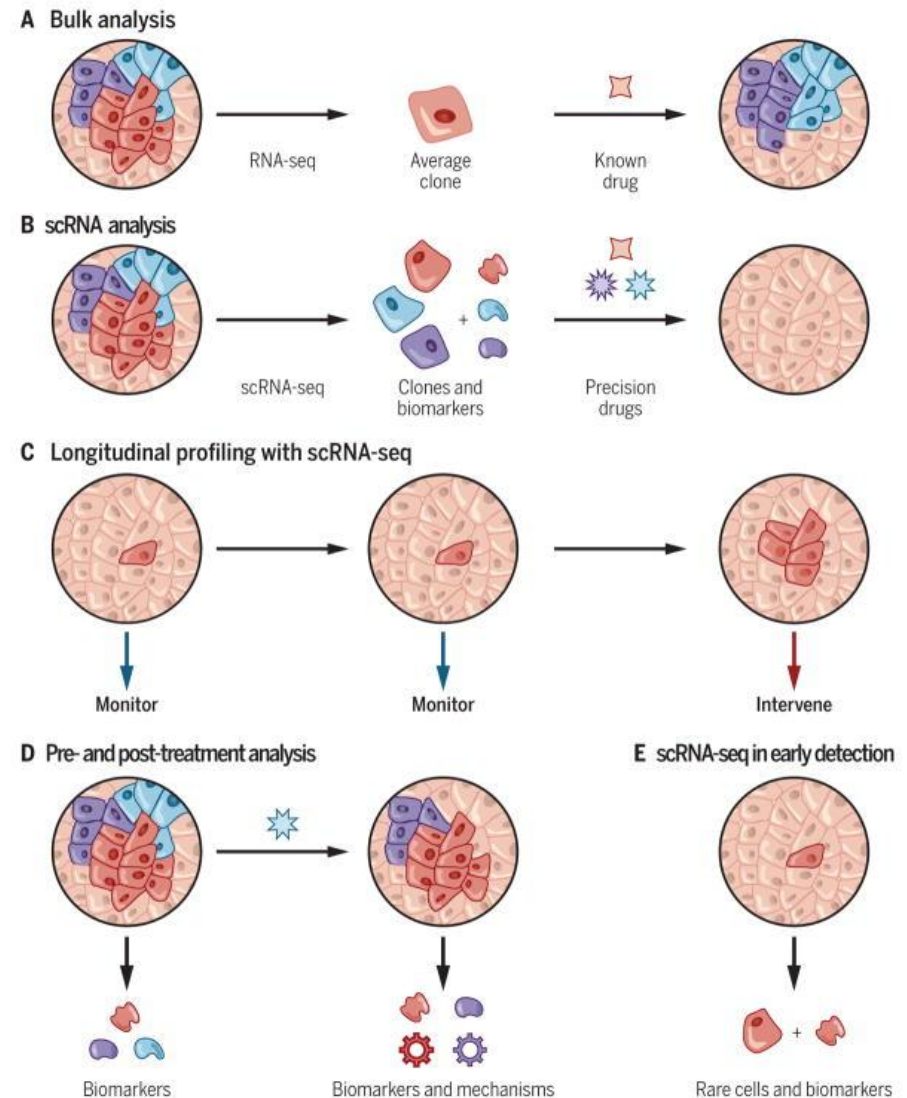
niko.beerenwinkel@bsse.ethz.ch (N.B.), angela.cluffi@chuv.ch (A.C.)

In Brief

HIV latency hampers HIV cure. The shock-and-kill strategy aims at reactivating HIV expression to purge the latent reservoir of HIV-infected cells. However, latently infected cells do not respond equally to stimulation. Golumbeanu et al. use single-cell RNA-seq to characterize cell heterogeneity and identify transcriptional features leading to reactivation success.

Cancer research: clinical applications

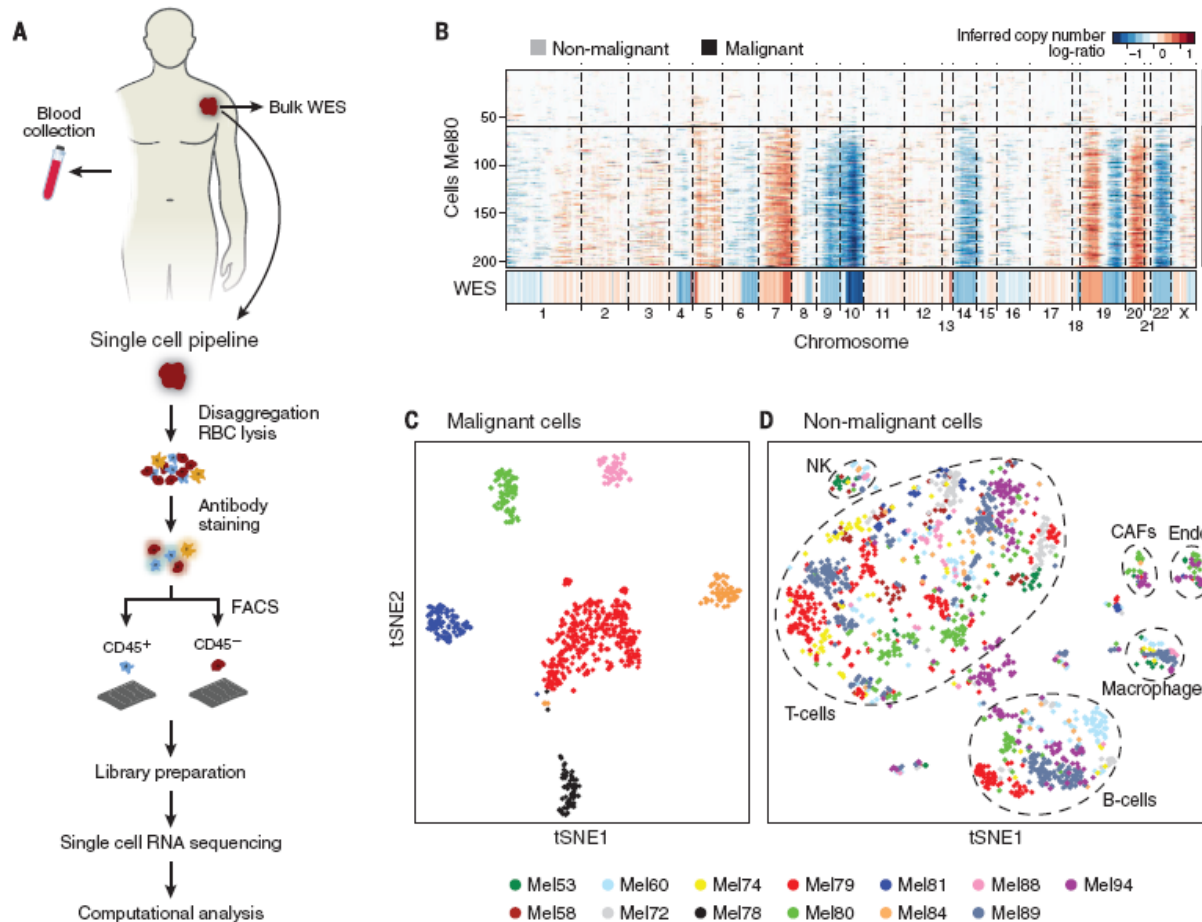
- scRNA-seq used for cellular heterogeneity studies
 - Liquid/solid tumors,
 - Early/metastatic, PDX,...
 - Tumor/microenvironment studies
- Clinical applications are still limited
- Potential use: measurement of minimal residual disease



Minimal Residual Disease

Tirosh, metastatic melanoma, Science 2016

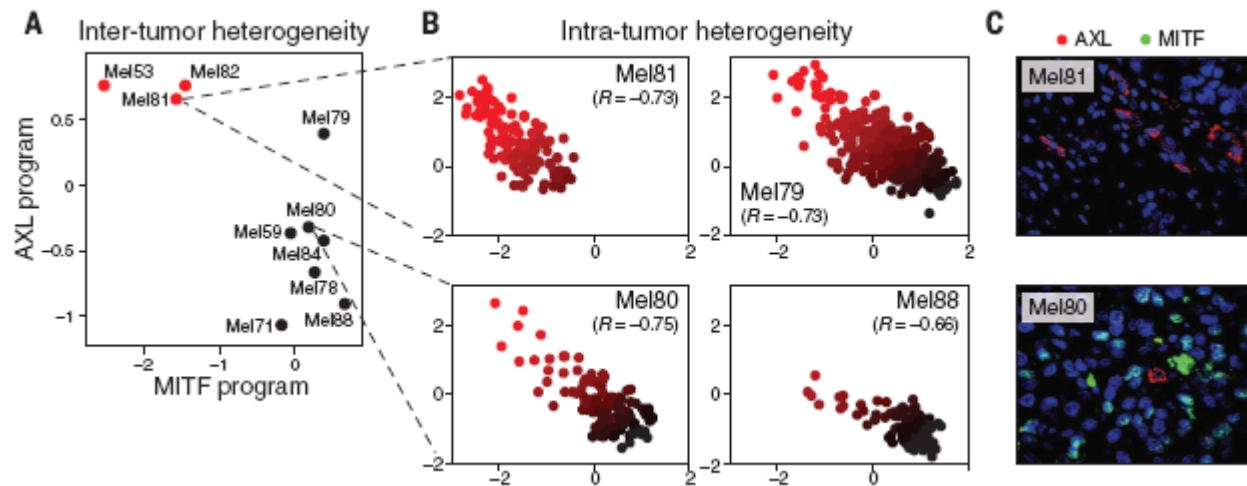
- Profiling of melanoma by scRNA-seq
 - Identify rare/hidden cells which may lead to relapse
 - Profiling of immune cells



InferCNV
TCR profiling

Intra-tumor heterogeneity

- Use genes correlated with firsts PCs to find gene programs involved with metastatic disease
- Find genes highly correlated with MITF -> MITF program
- Find genes negatively correlated with MITF -> AXL program
- Heterogeneity of the scores associated with these programs within / between tumors



- Large scale collaborative projects
- Provide complete maps of
 - Immune system
 - Entire organs (brain)
 - Entire organisms (nematostella,...)

ARTICLE

<https://doi.org/10.1038/s41586-018-0590-4>

Single-cell transcriptomics of 20 mouse organs creates a *Tabula Muris*

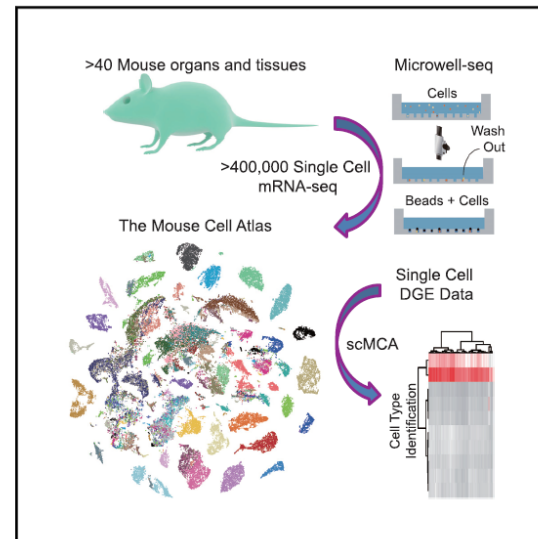
The Tabula Muris Consortium*

Resource

Cell

Mapping the Mouse Cell Atlas by Microwell-Seq

Graphical Abstract



Authors

Xiaoping Han, Renying Wang, Yincong Zhou, ..., Guo-Cheng Yuan, Ming Chen, Guoji Guo

Correspondence

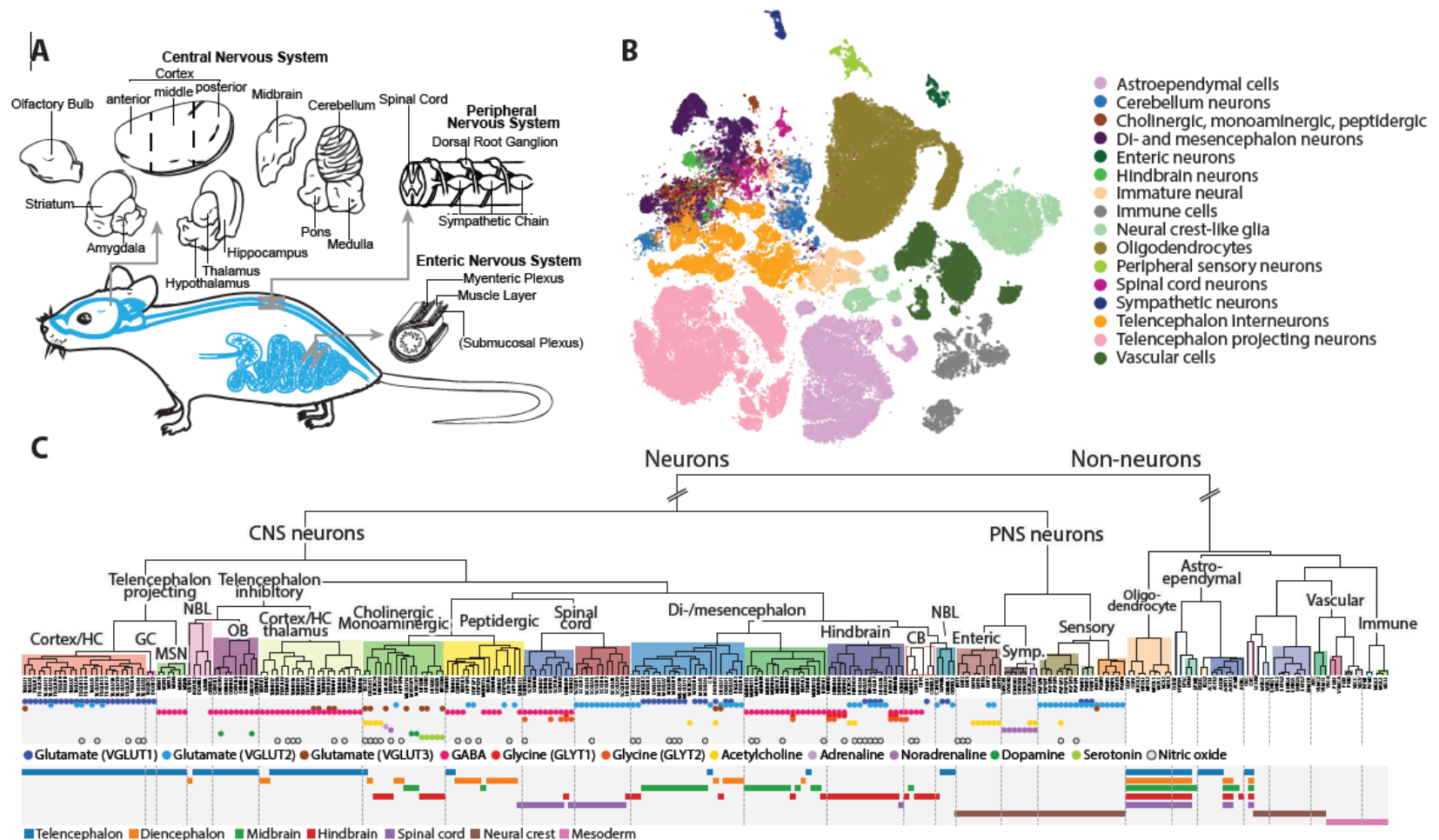
xhan@zju.edu.cn (X.H.),
ggj@zju.edu.cn (G.G.)

In Brief

Development of Microwell-seq allows construction of a mouse cell atlas at the single-cell level with a high-throughput and low-cost platform.

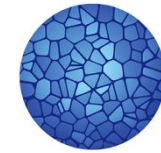
MCA browser
<http://bis.zju.edu.cn/MCA/>

Mouse Brain Atlas



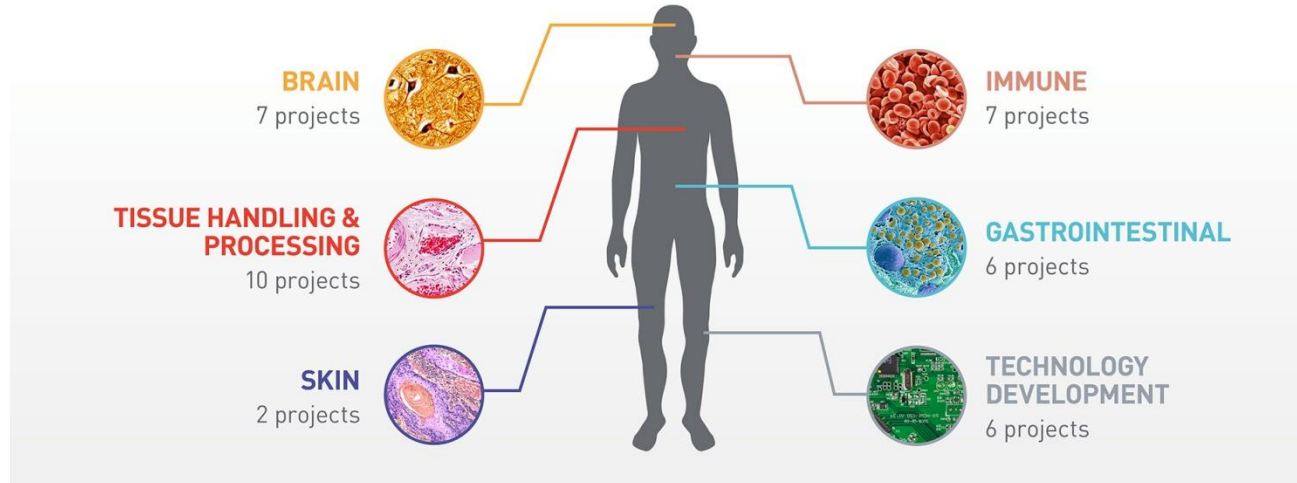
<http://mousebrain.org/>

Zeisel, Cell 2018

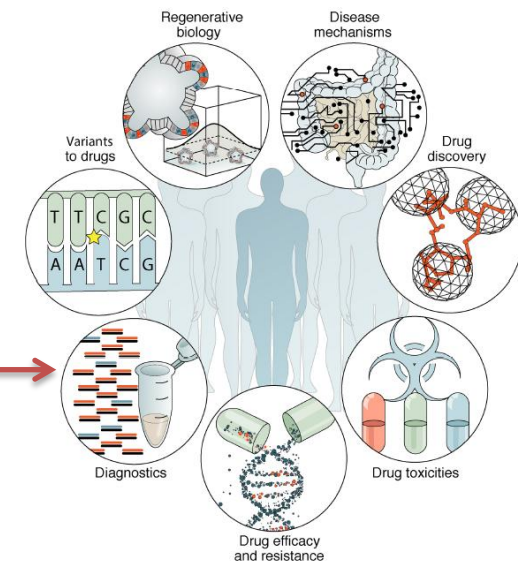


MAPPING THE BASIC UNITS OF LIFE

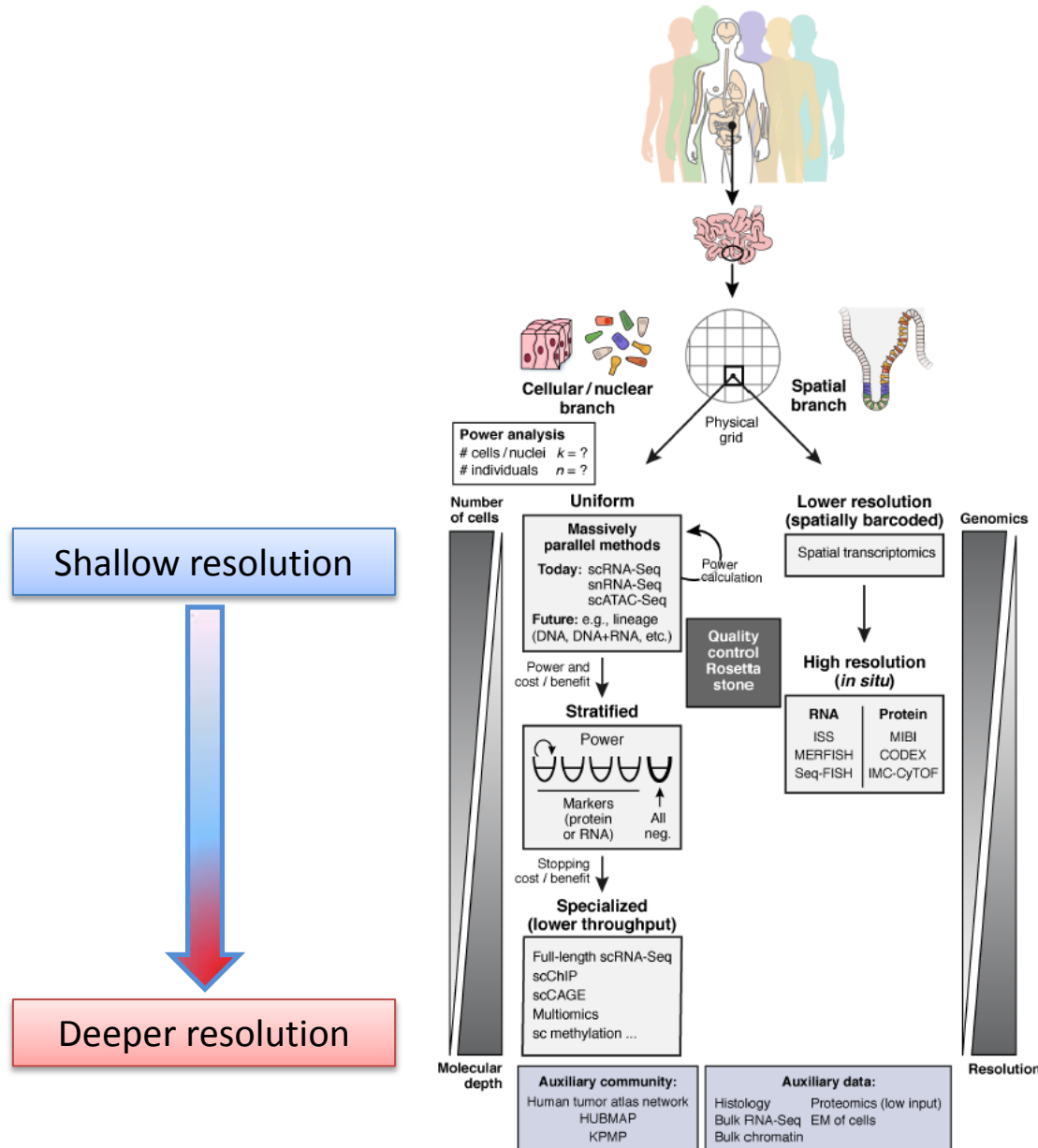
CZI proudly supports **38 new projects** in these six areas for the **Human Cell Atlas**.



- Every cell type in the body
- First: define how to proceed
 - Best experimental practice / organ
 - Best bioinformatics methods
- Data will be made available to all



Different layers of information



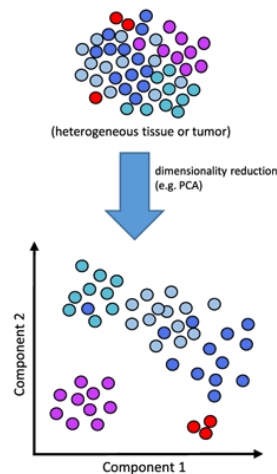
Take Home Message

- These atlases are great resources for your projects
- Interfaces / tools are provided for browsing/ data access
- WARNING: be aware of datasets limitations (quality of the data, sequencing depth, techno used...)
- Applications:
 - Reference to type cells in your project
 - Automated tools to phenotype with confidence your cell in a very near future?
 - Web based interface -> everybody can use them

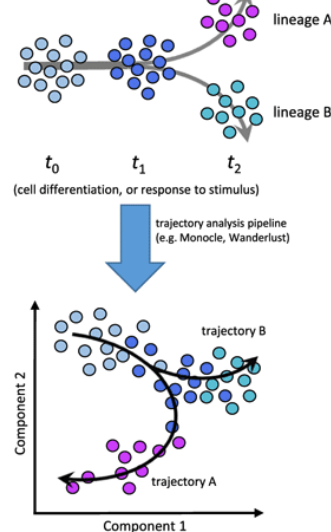
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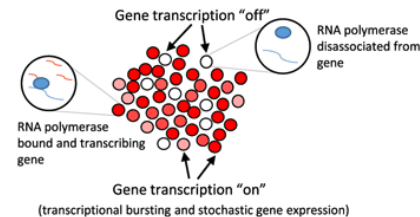
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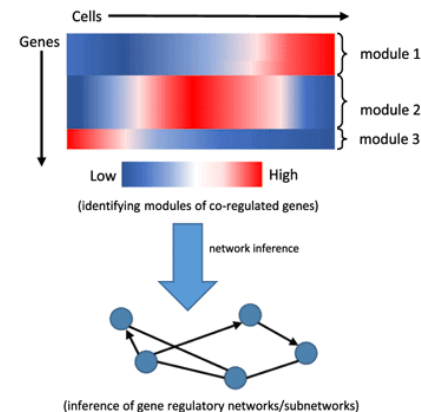
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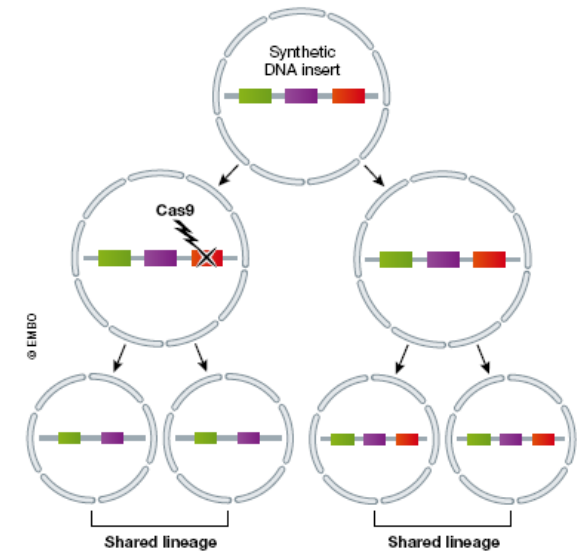


d) Network inference



Trajectory Analysis /Cell State and Cell Fate

- Embryogenesis/Development studies
 - Use genome editing to track cell lineage
- Cell fate decisions
 - How does a cell decide her fate?
 - Cancer cells evolution and clonal analysis
 - Variation in gene expression and stochasticity in cell decision processes



Griffiths JA, 2018

Trajectory inference from scRNAseq

2498 Robrecht Cannoodt et al.

Eur. J. Immunol. 2016. 46: 2496–2506

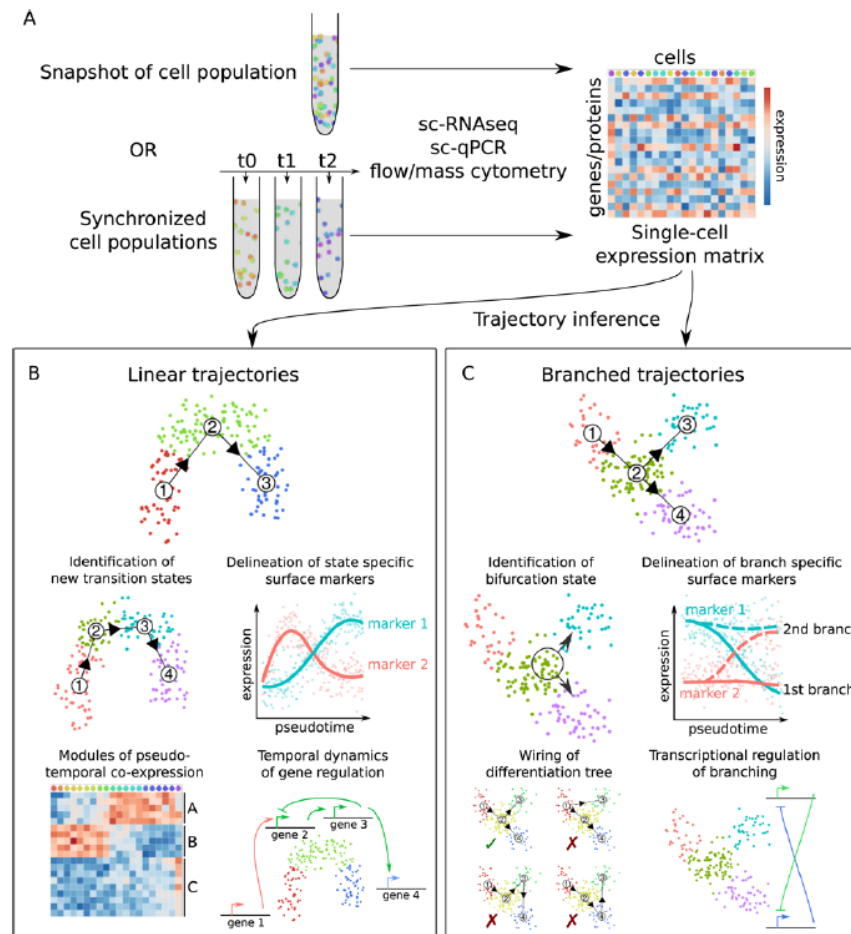
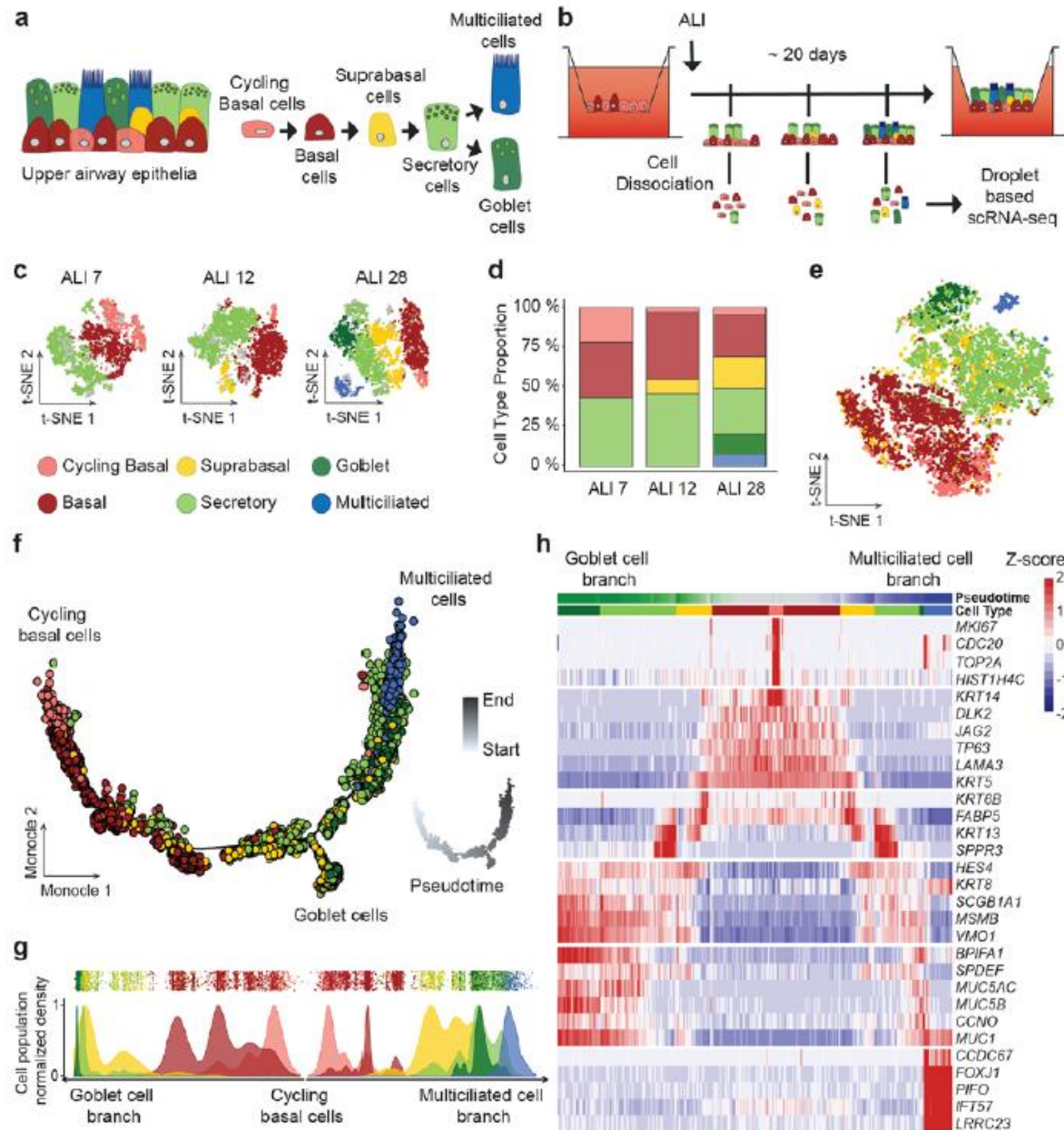
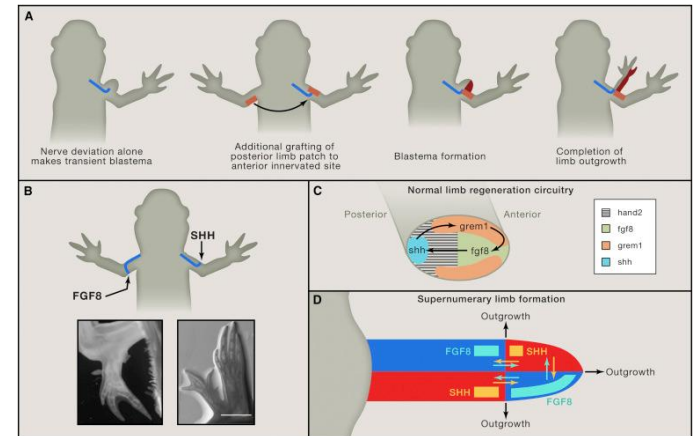
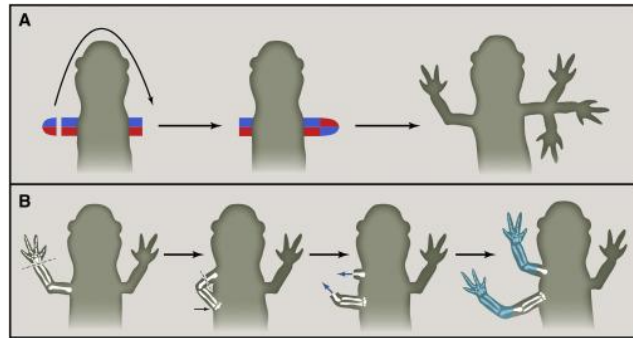


Figure 1. Applications of single-cell trajectory inference methods. (A) Single-cell data appropriate for TI can be both obtained from an unsynchronized population of single cells (snapshot data) but also from synchronized cell populations. Single-cell expression data can be obtained genomewide (sc-RNAseq), for a subset of genes (sc-qPCR) or for a set of proteins (flow/mass cytometry). (B) Linear trajectories can be used to find new transition states, try to find new markers to better separate transition states, identify coexpression modules, and investigate the dynamics of gene regulation underlying the dynamic process. (C) In addition to all applications of linear trajectories, branched trajectories can also be used to identify bifurcation states, find protein markers to better separate specific branches of the developmental tree, identify how cells move from one transition state to another in vivo, and understand how the commitment to a particular branch works at the level of the gene regulatory network.

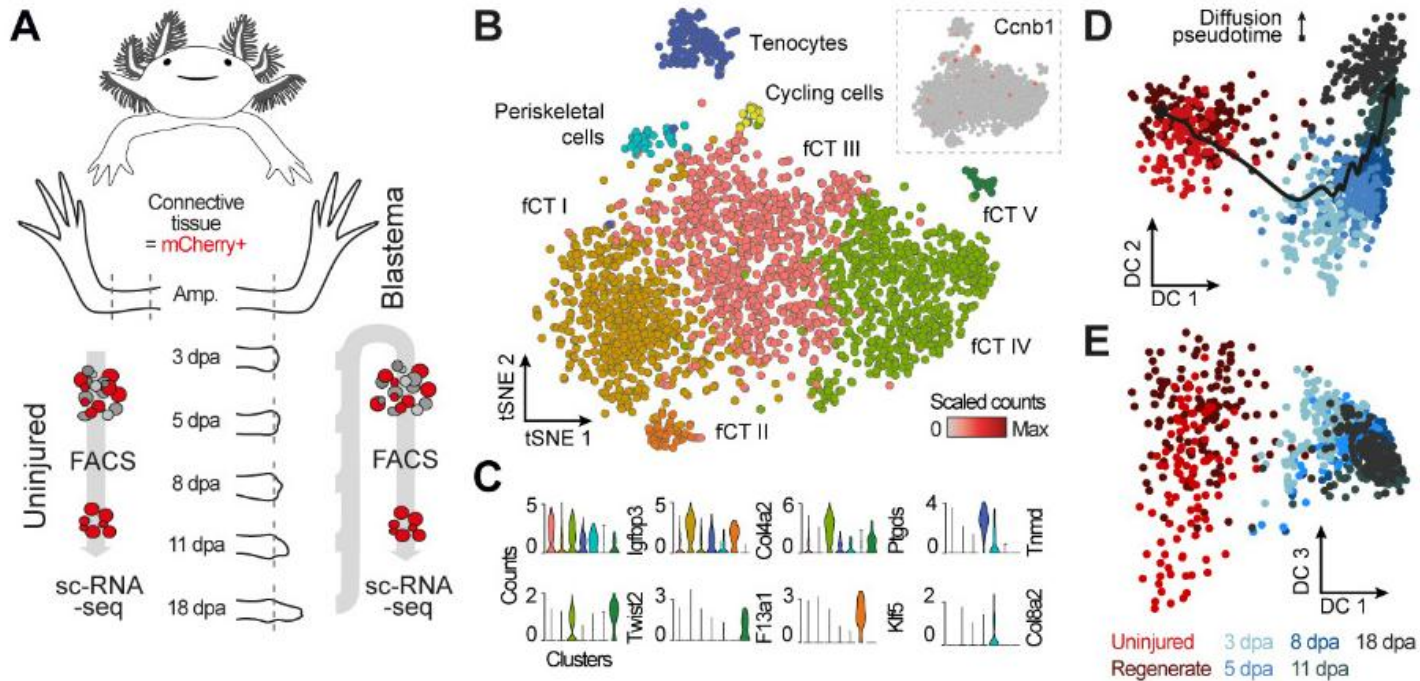
Airway epithelium regeneration study



Regeneration of Limbs: Axolotl model



Tanaka, Cell 2016

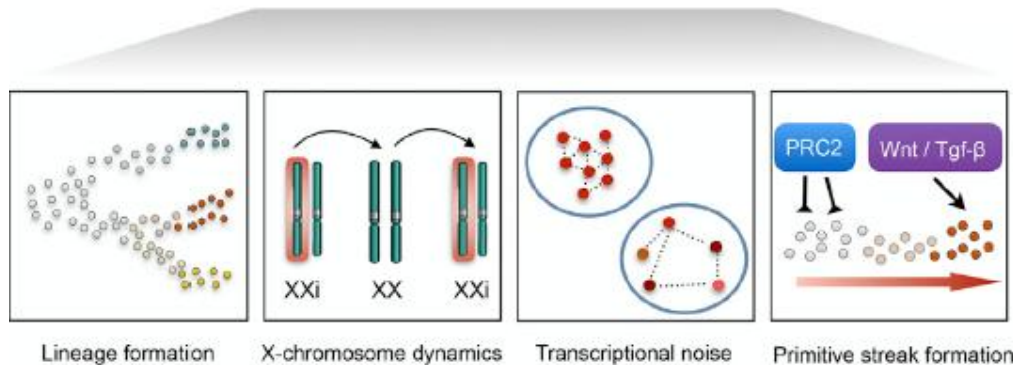
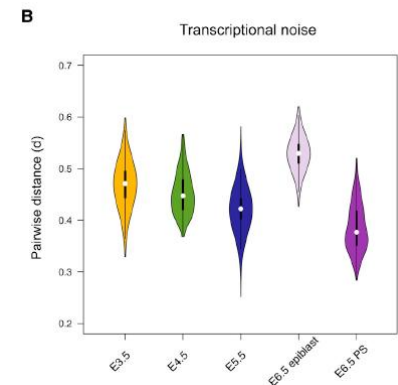
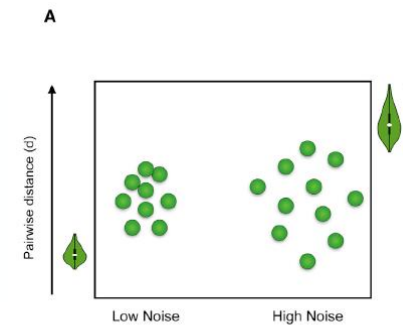
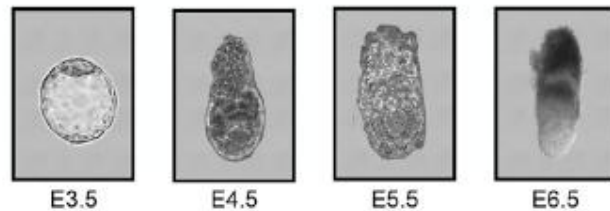


Gerber, Science 2018

Cell Reports

Single-Cell Landscape of Transcriptional Heterogeneity and Cell Fate Decisions during Mouse Early Gastrulation

Resource

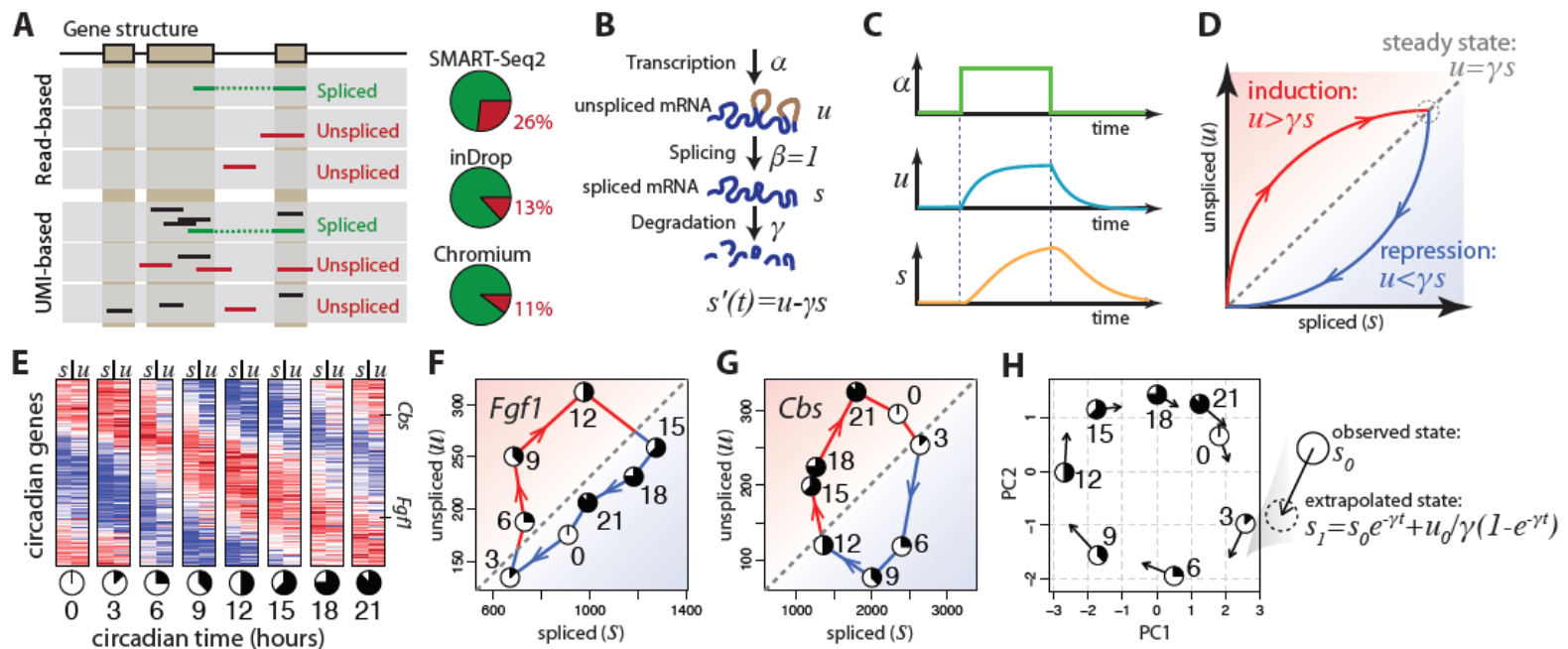


RNA Velocity

RNA velocity in single cells

Gioele La Manno, Ruslan Soldatov, Hannah Hochgerner, Amit Zeisel, Viktor Petukhov, Maria Kastriiti, Peter Lonnerberg, Alessandro Furlan, Jean Fan, Zehua Liu, David van Bruggen, Jimin Guo, Erik Sundstrom, Goncalo Castelo-Branco, Igor Adameyko, Sten Linnarsson, Peter Kharchenko

doi: <https://doi.org/10.1101/206052>



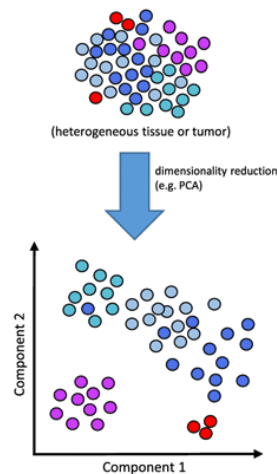
<http://velocyto.org/>

Nature, 2018

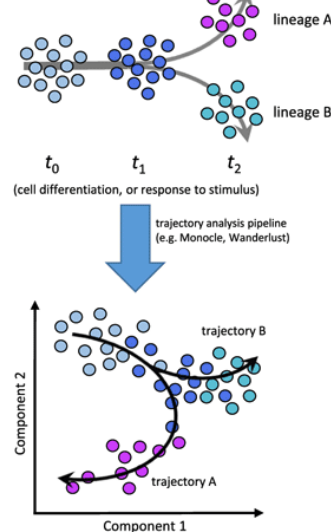
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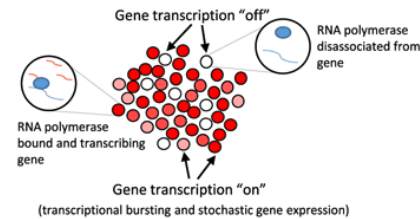
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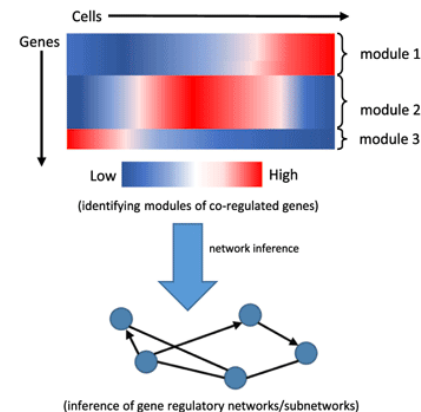
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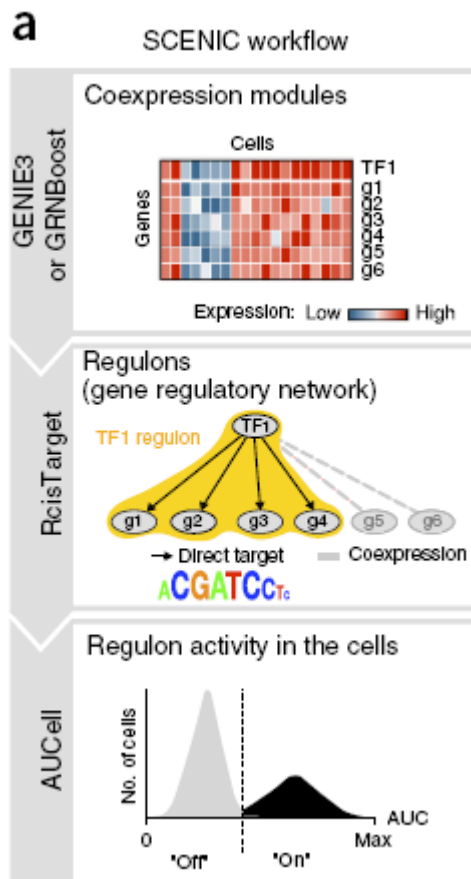


d) Network inference



SCENIC: single-cell regulatory network inference and clustering

Sara Aibar^{1,2}, Carmen Bravo González-Blas^{1,2},



Caveats:

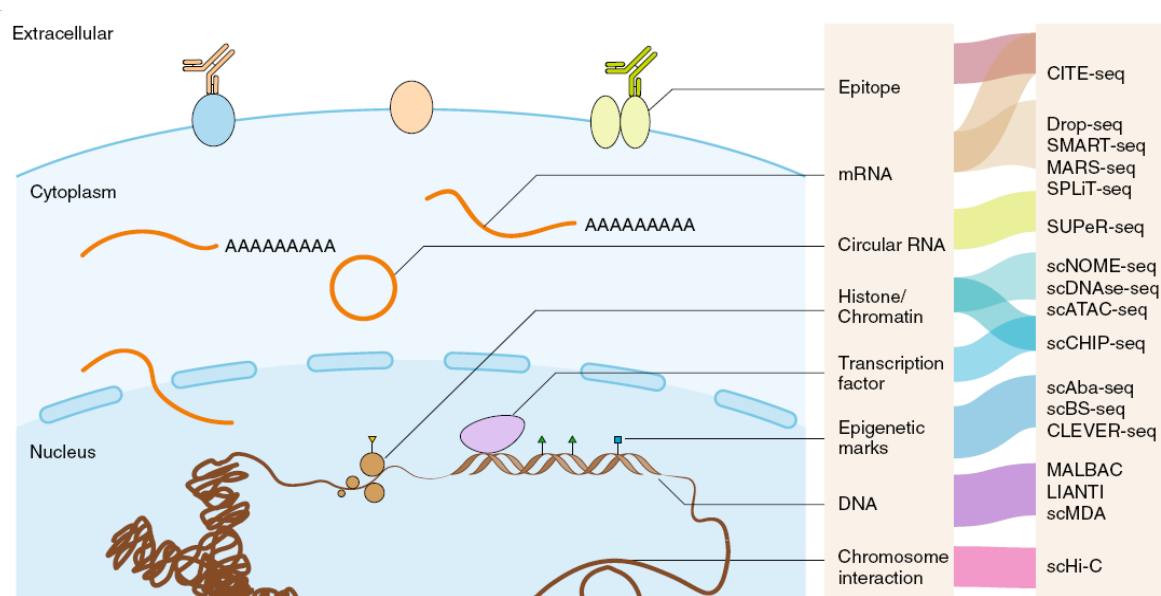
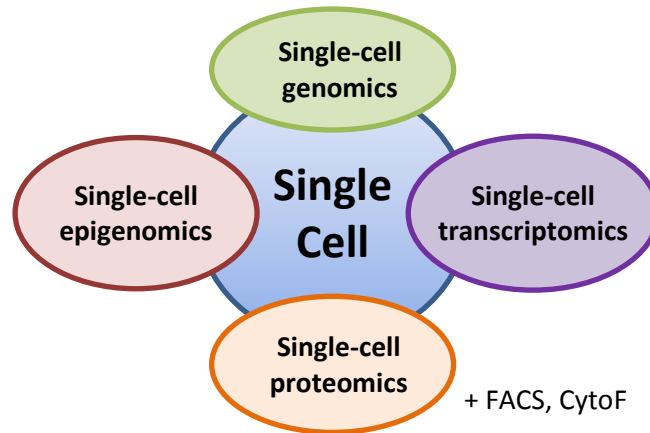
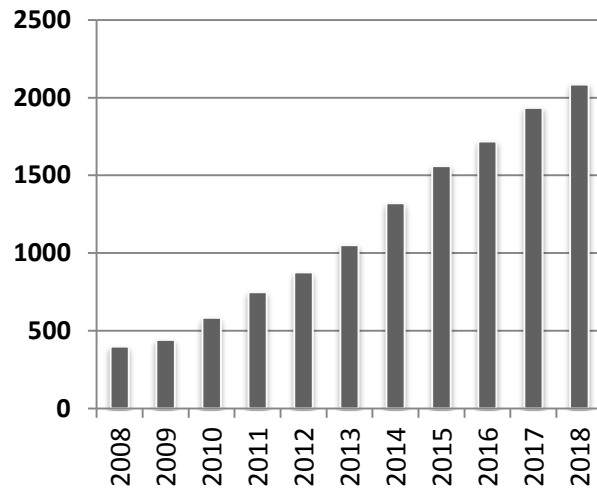
- Low sequencing depth
- Drop-outs
- > Difficult to generate robust networks

Future Directions

Integration of single cell OMICS

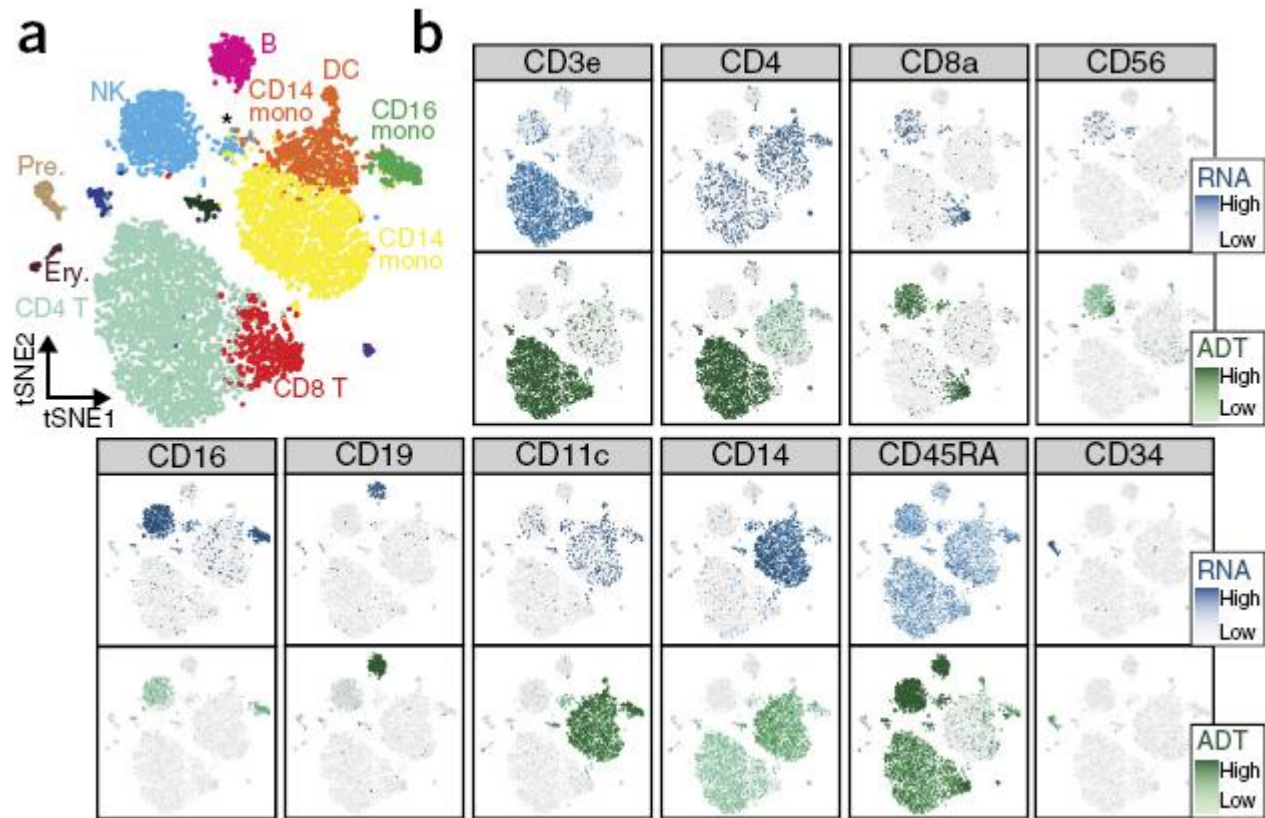
What type of profiling?

Pubmed counts for single cell seq



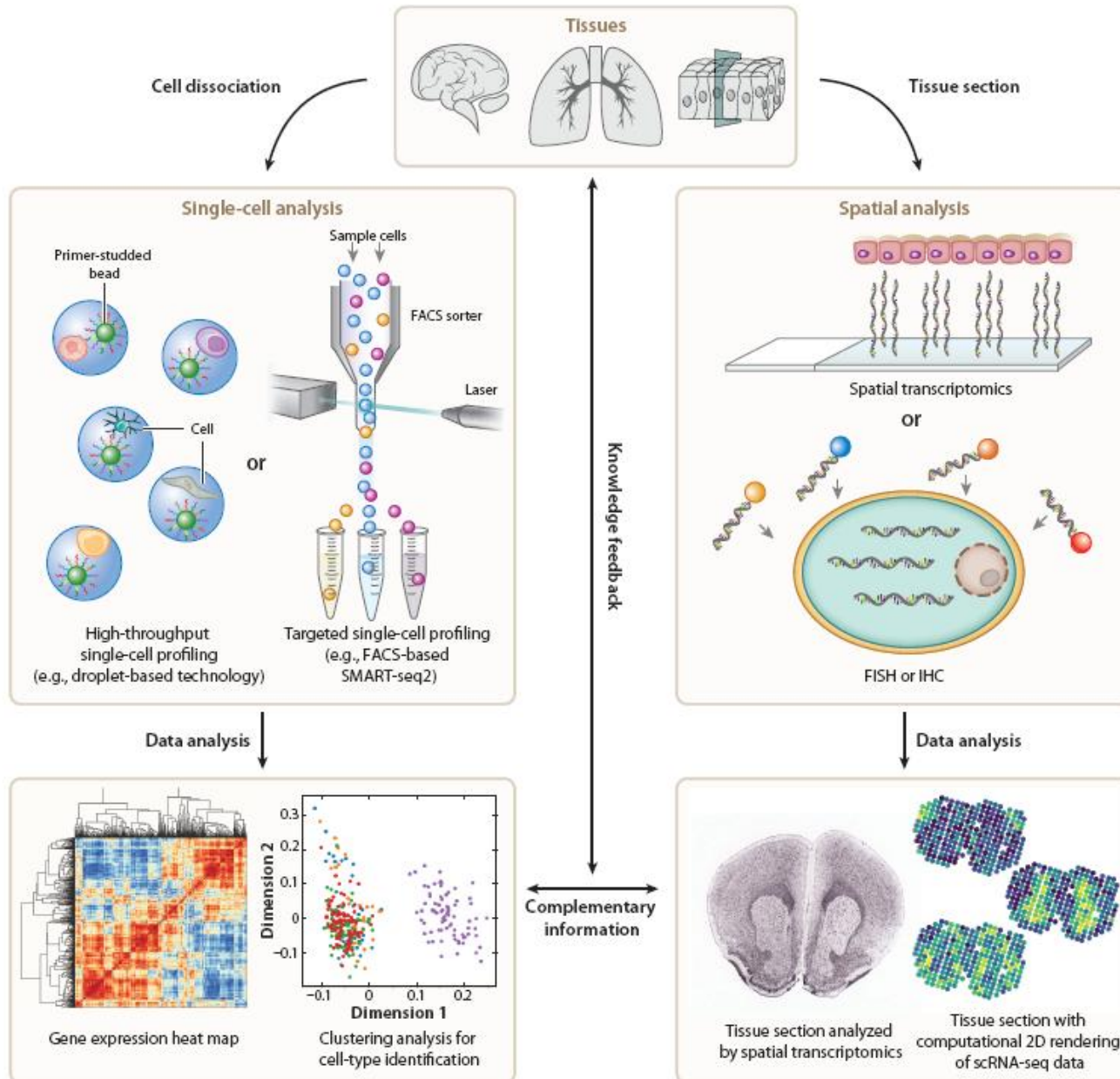
CITE-seq

Combine cell surface markers + single cell RNAseq



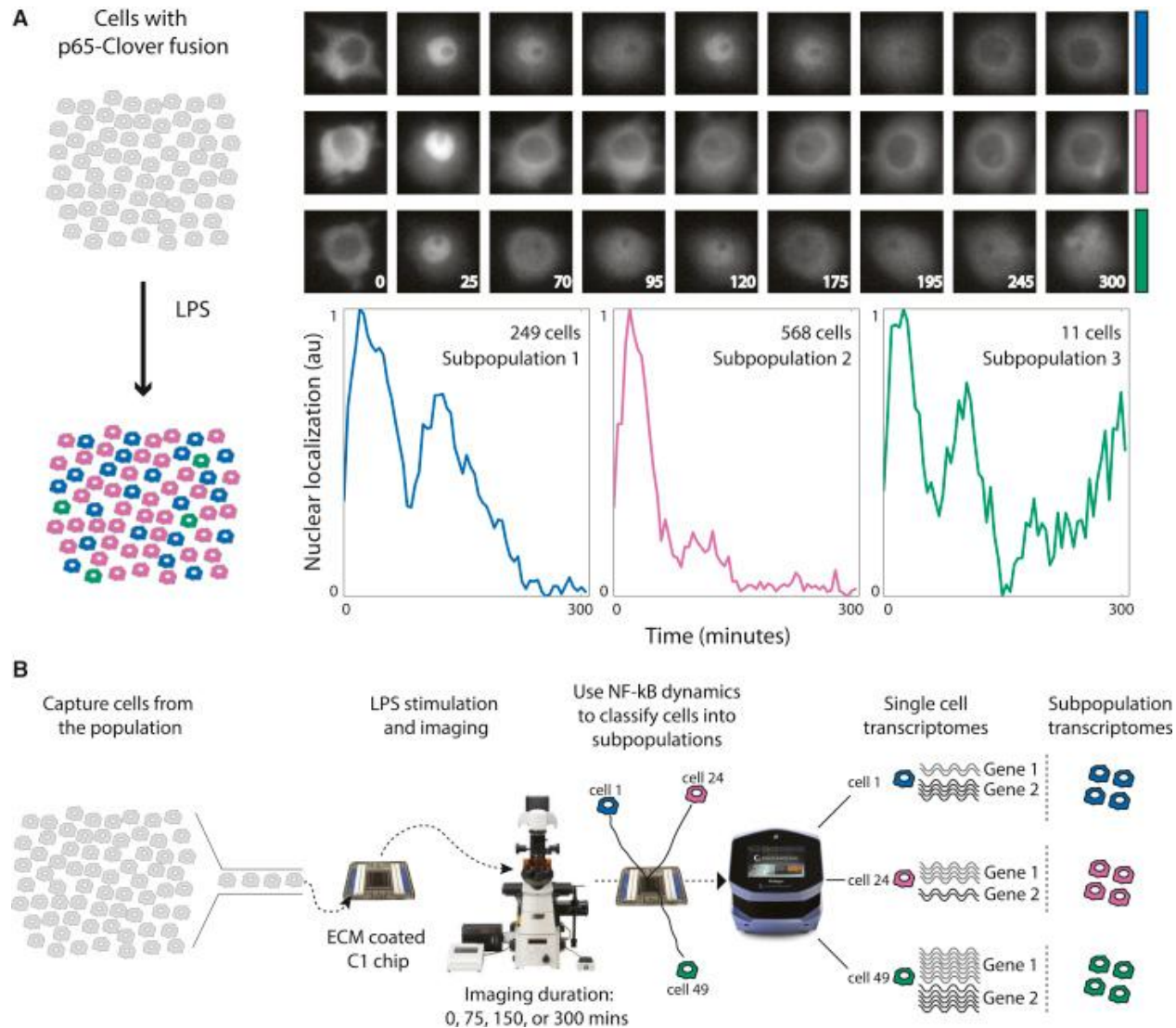
Stoeckius, Nat Med 2017

Spatial Transcriptomics




Live cell imaging + scRNA-seq

Lane K, Cell Syst 2017

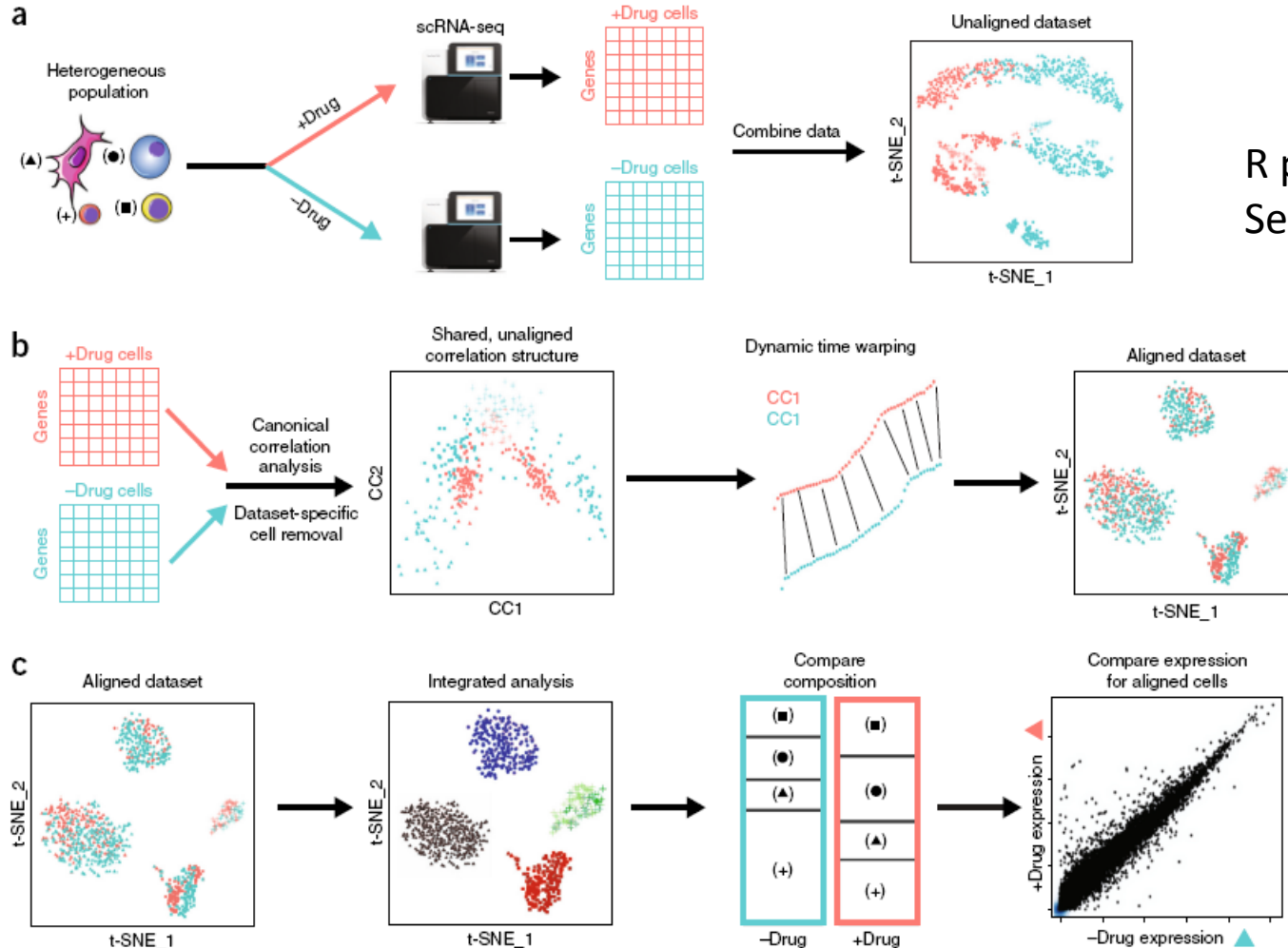


Data Integration Methods Required

Integrating single-cell transcriptomic data across different conditions, technologies, and species

Andrew Butler^{1,2}, Paul Hoffman¹, Peter Smibert¹, Efthymia Papalexi^{1,2} & Rahul Satija^{1,2} 

Nature Biotech 2018



R package
Seurat

- Non-linear transformation of cells in different proportions
- Aligns datasets from different technologies and species