Mass General Brigham

Clinical Applications of Single cell-RNA sequencing

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Division of Pulmonary and Critical Care Medicine
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Brigham and Women’s Hospital
Harvard Medical School

4/2023

HARVARD
MEDICAL SCHOOL

Agenda

1. What is single cell RNA seq?

2. Steps of performing single cell RNA seq

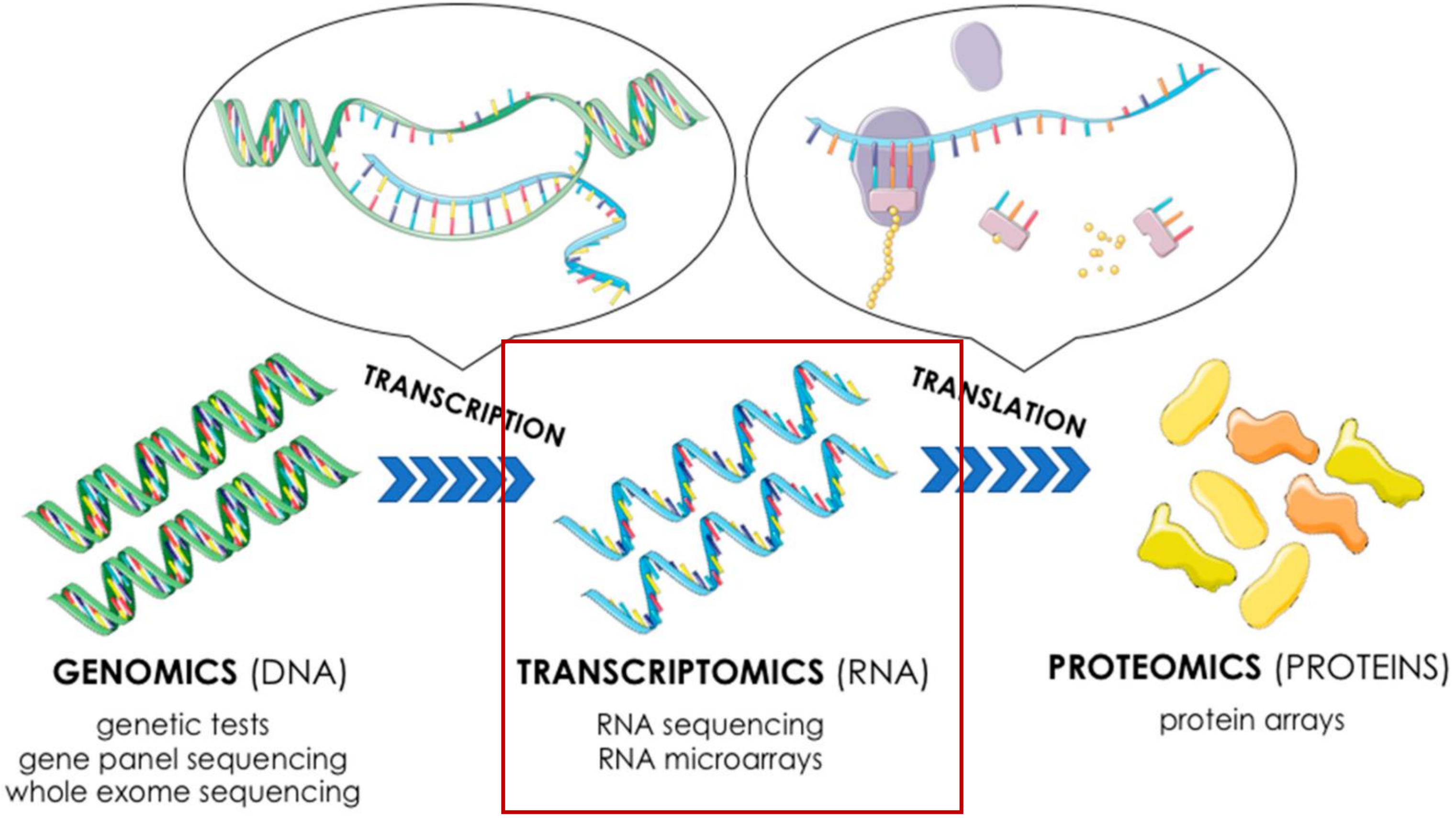
3. Application of Single cell RNAseq

4. Limitations and Future directions

What is single cell RNA-seq?

단일세포 전사체 기술이란?

전사체 연구와 임상적 적용

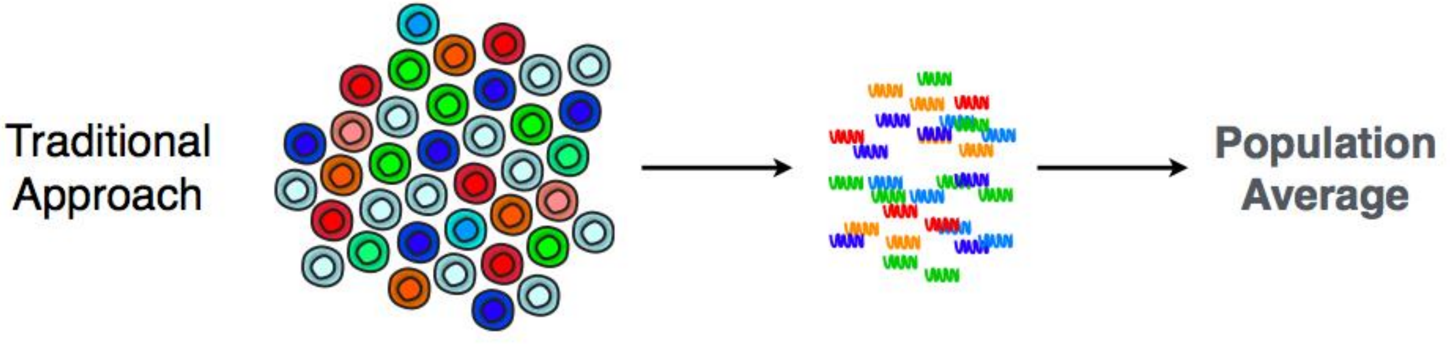


Supplitt et al. Int. J. Mol. Sci 2021

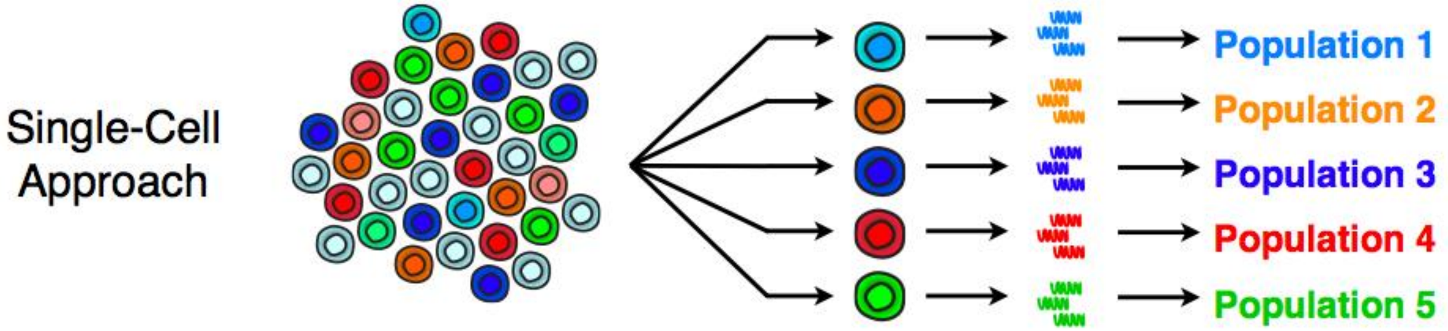
- 발달 단계, 병태생리학적 변화, 환경적 요인에 의한 전반적인 유전자 발현 변화 연구
- Good dynamic range
- Easy to capture

- 폐암의 분류, 조기 진단
- Asthma, COPD, IPF의 분자적 기전
- 감염 (TB, COVID19)의 진단과 예후 예측

Bulk vs. Single cell RNA sequencing



전체 조직의 평균적인
유전자 발현 변화
~가장 많은 비중을 차지하는
세포



개별 세포의
유전자 발현 변화

Adapted from Dan Wagner HMS

What can we learn from single cell–RNAseq?

1. Cell types/states

2. Cell transition

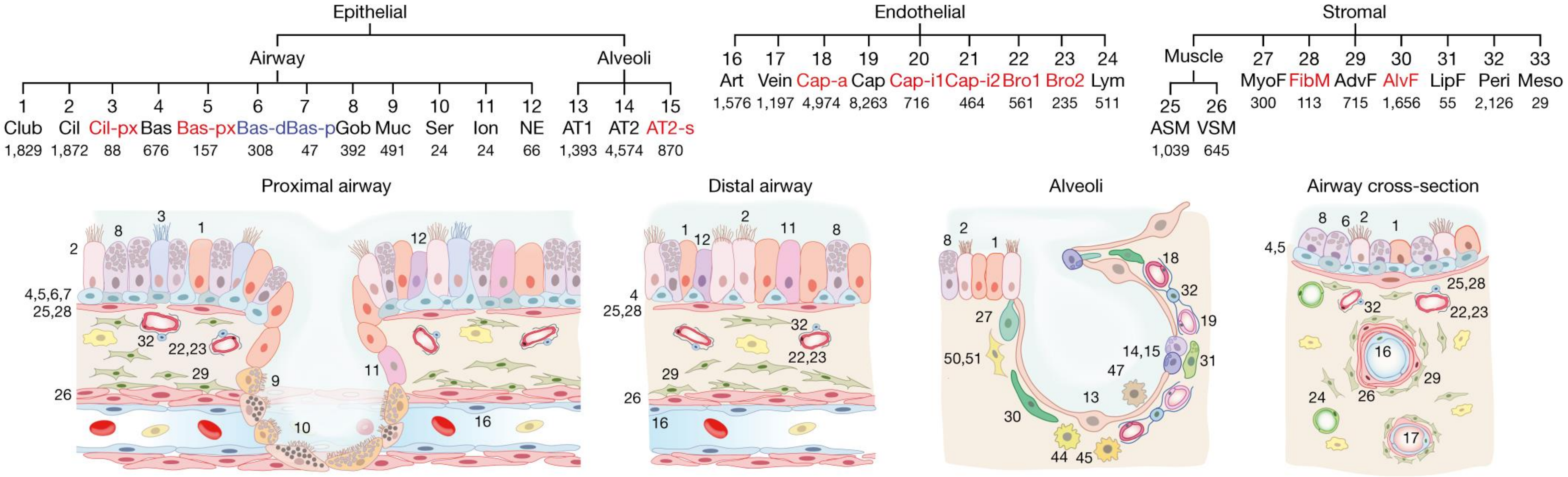
3. Variability of gene expression

4. Covariation

5. Cell-cell interaction

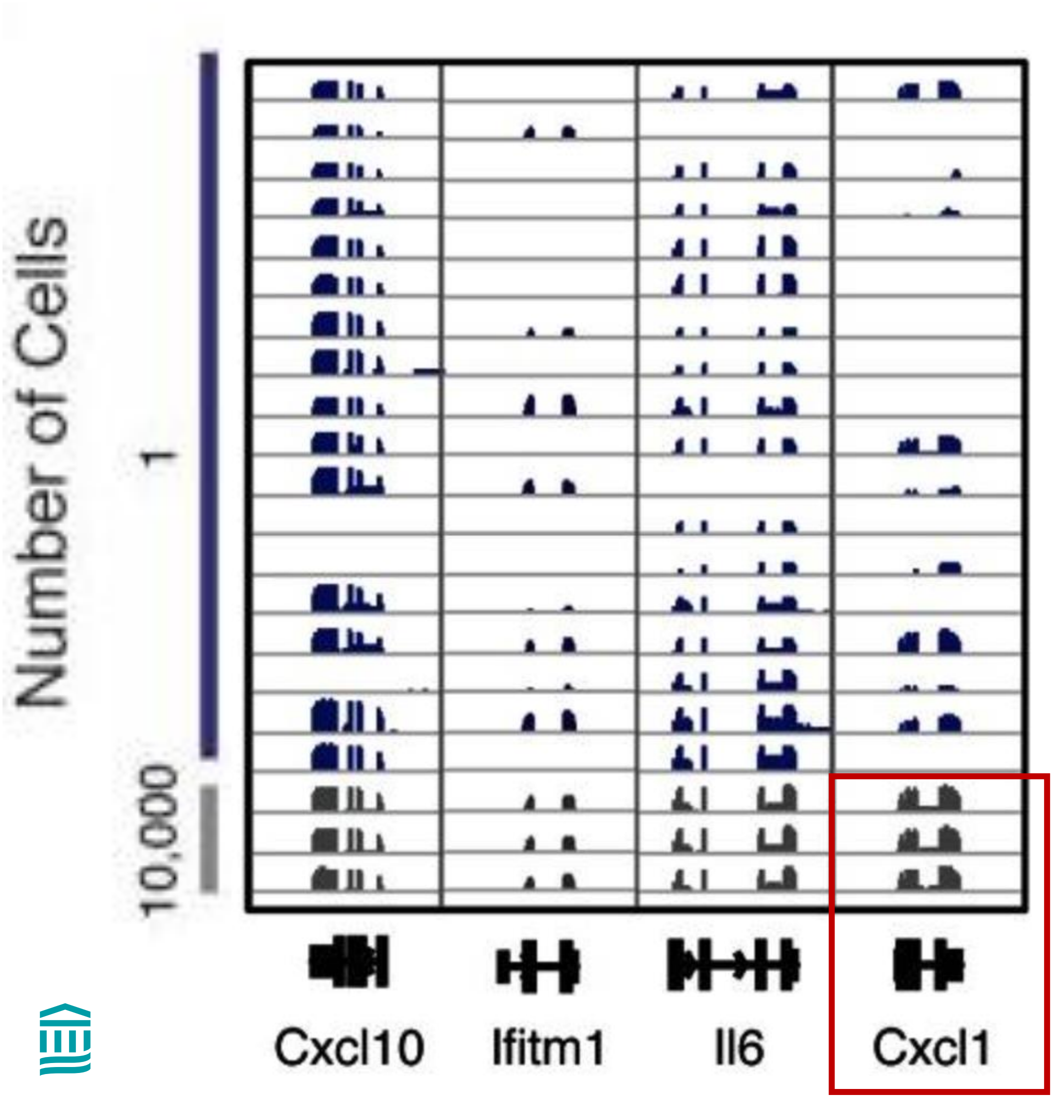
What can we learn from single cell–RNAseq?
Cell types/states

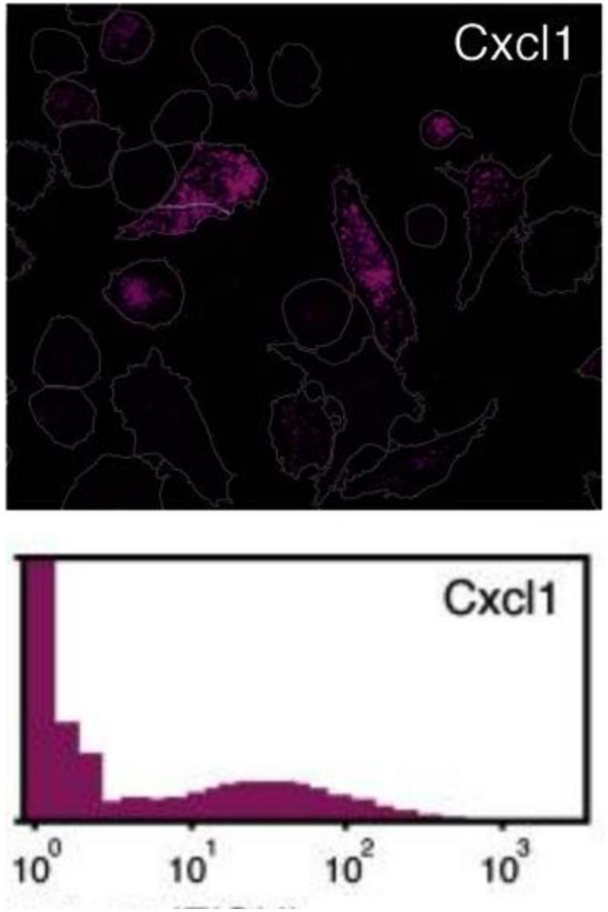
- 사람의 몸: 30조 (30,000,000,000,000) 세포
- 교과서: 200개의 주요 세포 종류
- 폐: 60종 이상



Travaglini et al. Nature 2020

What can we learn from single cell–RNAseq?
Variability in gene expression

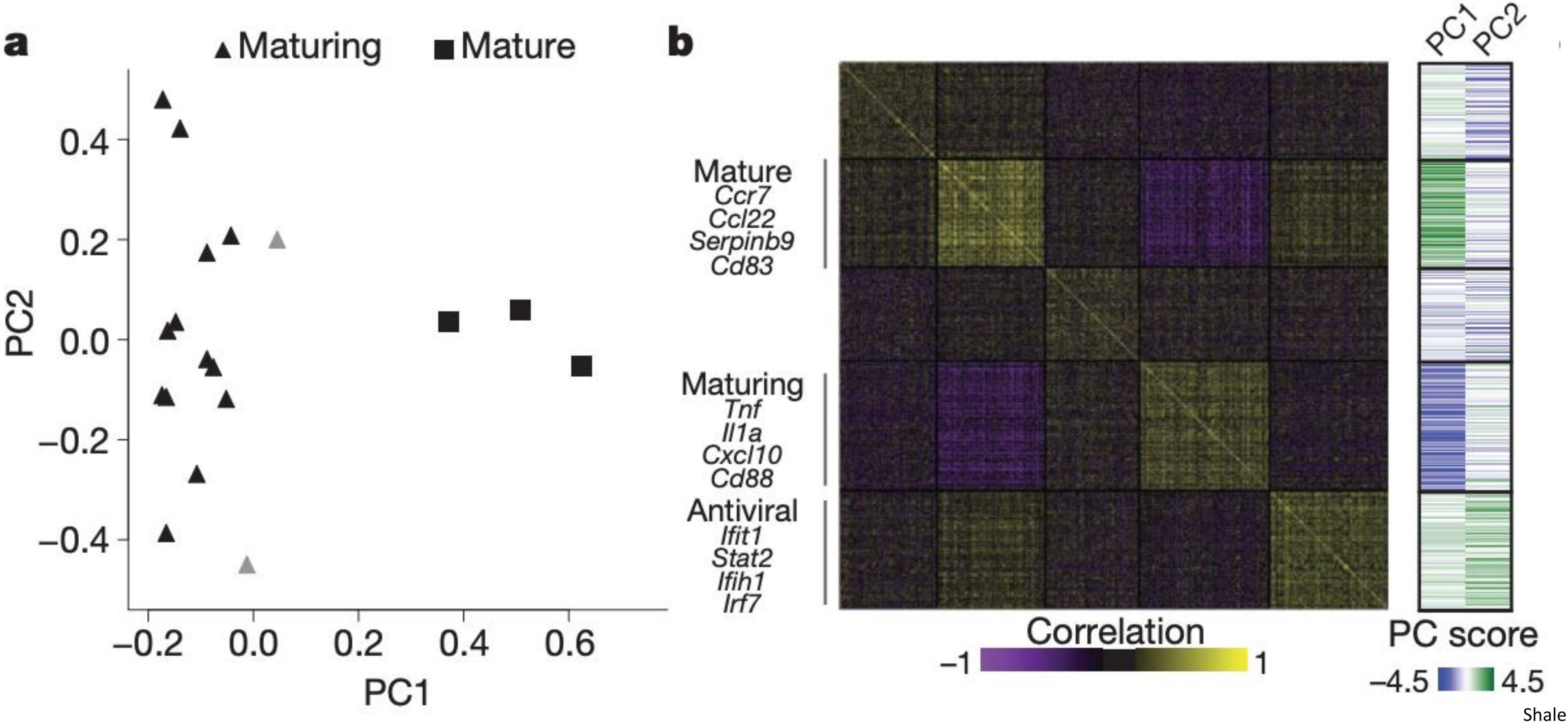




Shalek*, Satija* et al. Nature 2013

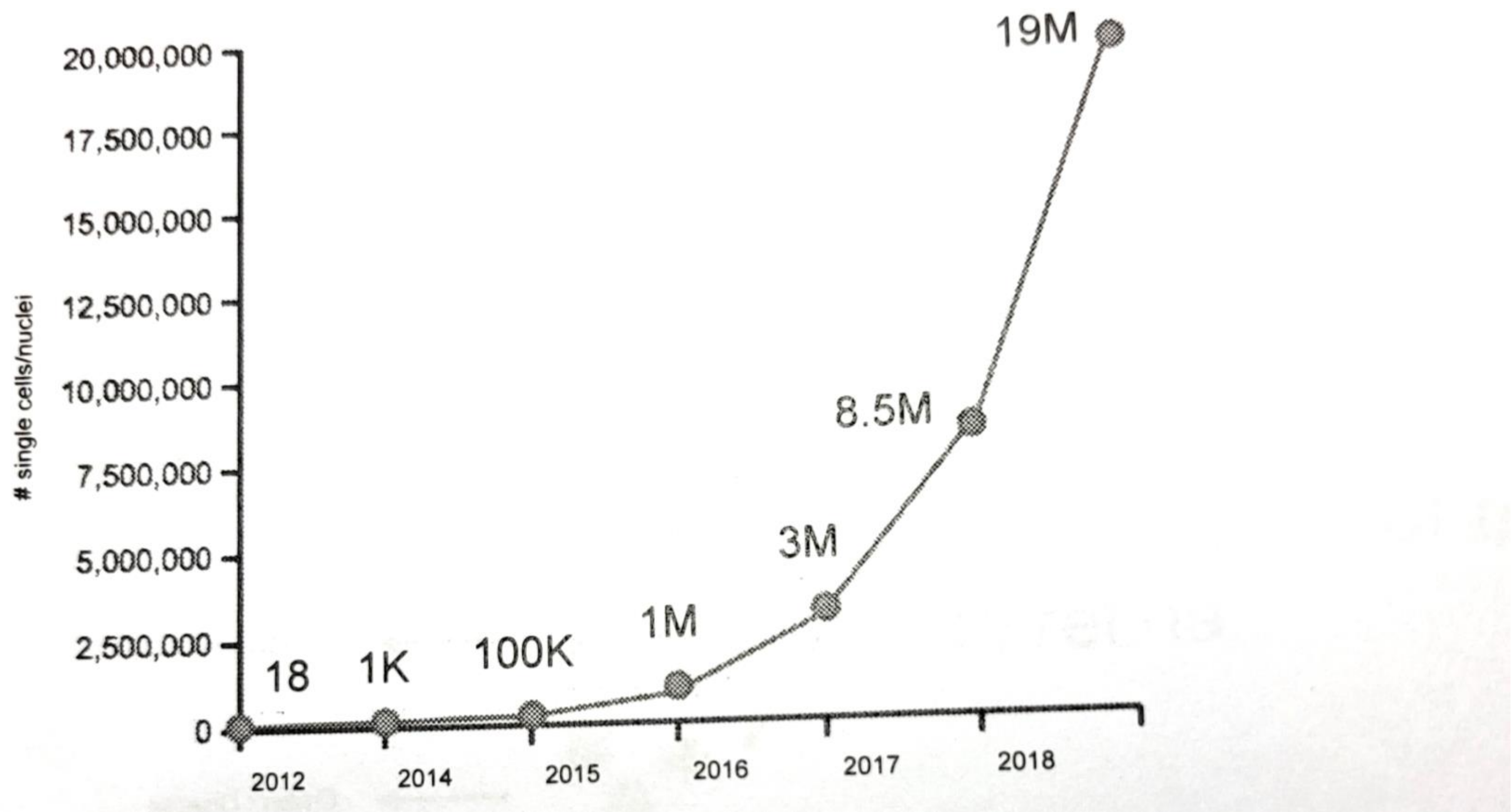
What can we learn from single cell–RNAseq?

Biology from patterns in gene expression covariation



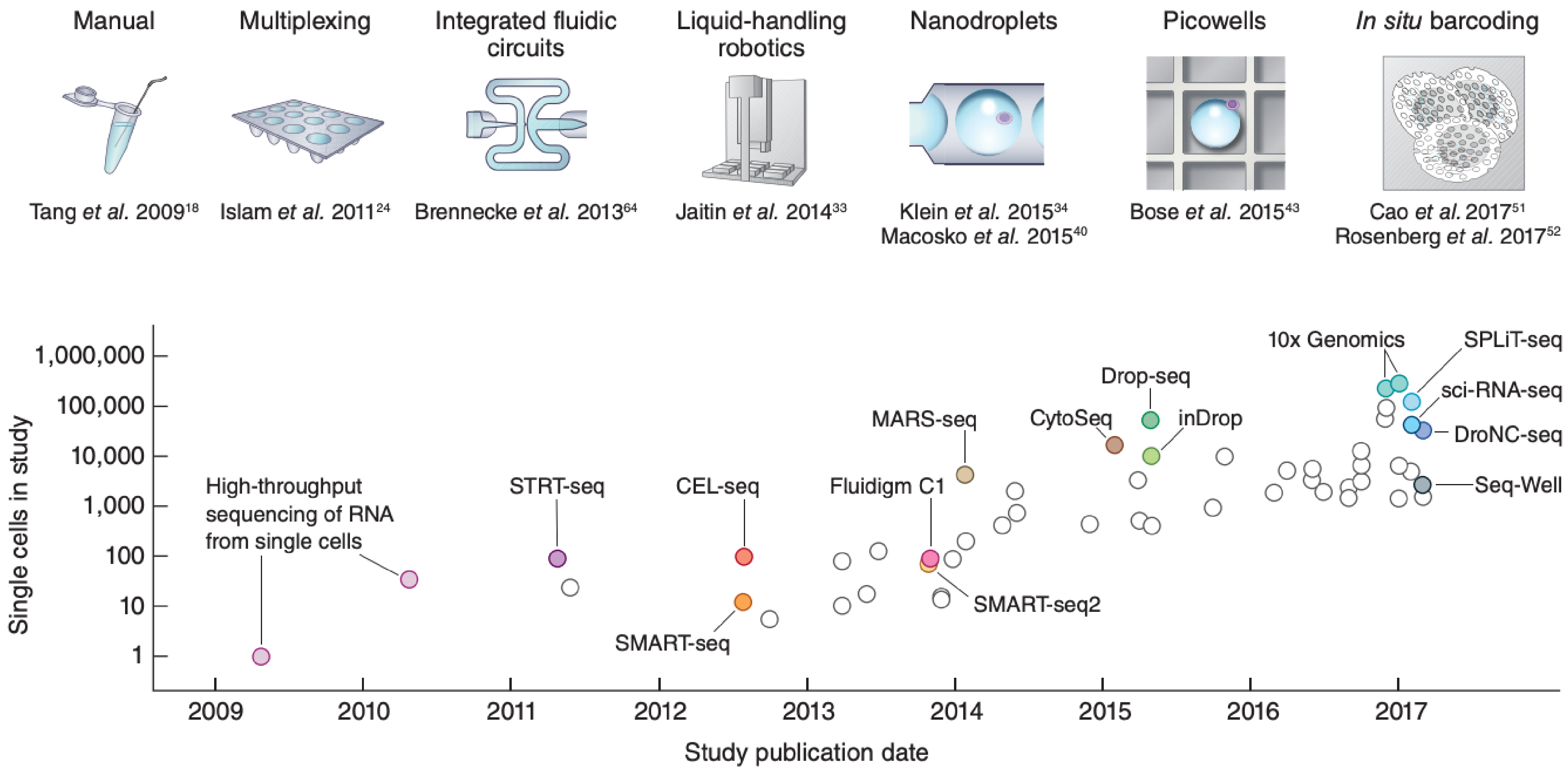
Shalek*, Satija* et al. Nature 2013

단일세포 전사체 연구의 폭발적 성장



Courtesy of Alex Shalek, Broad Institute

단일세포 전사체 연구의 기술적 발전

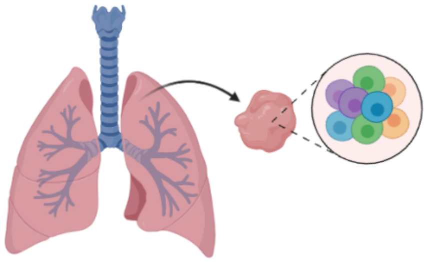


Svensson et al. Nature Protocols 2018

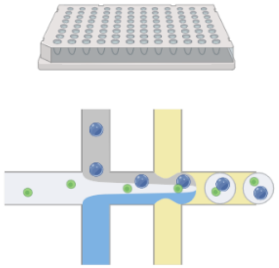
Steps of performing single cell RNAseq

Steps of performing single cell RNA-seq
Experimental pipeline

① Sample preparation



② Cell capture and barcoding



③ Library Prep

full -length vs.
3'/5' end tagging

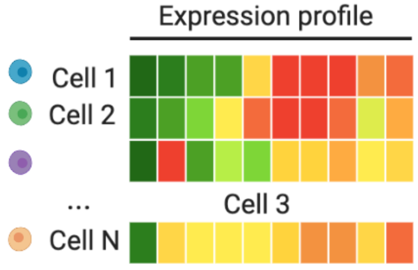
PCR vs. in vitro transcription

④ Single-cell sequencing

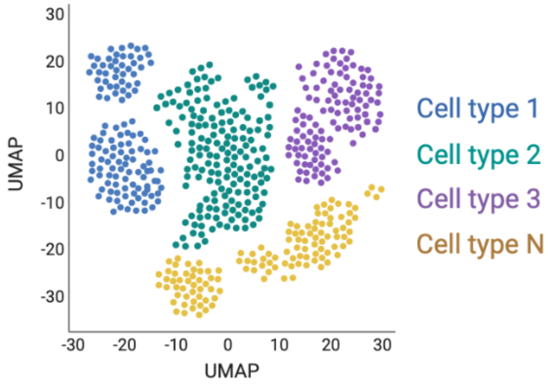
⑤ Preprocessing

Alignment
Quality control
Filtering
Normalization

⑥ Single-cell expression profile



⑦ Clustering & cell type identification
Downstream analysis

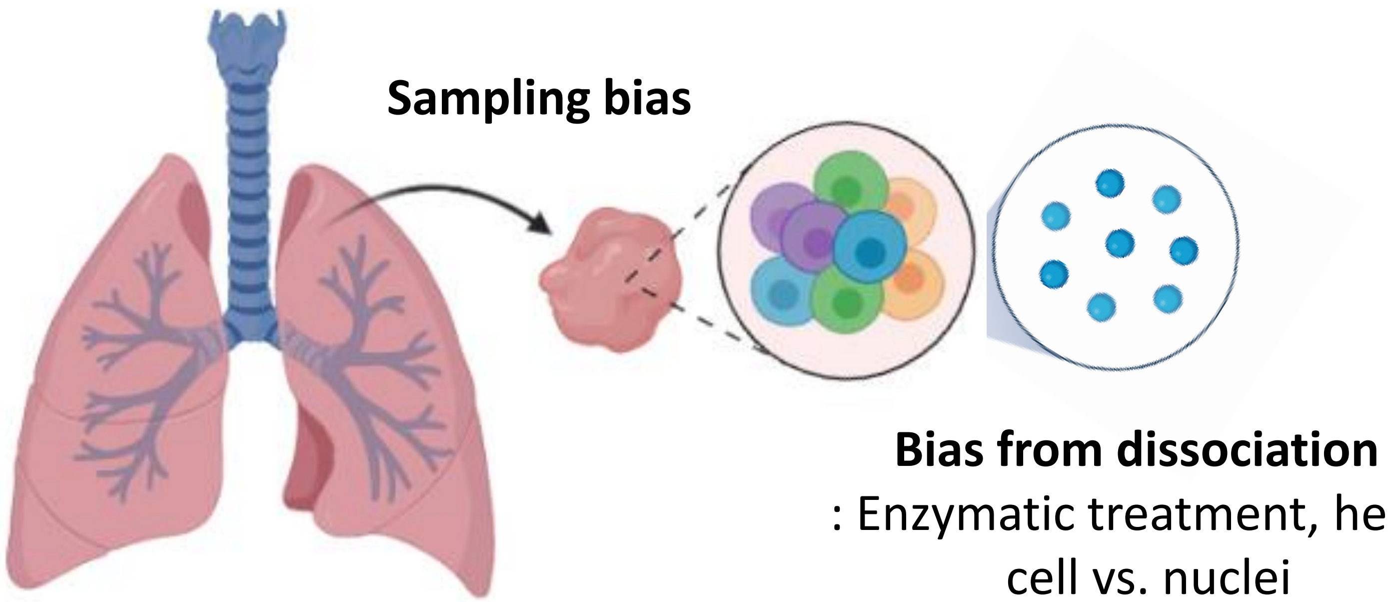


Created with BioRender.com

Steps of performing single cell RNA-seq
Sample preparation

① Sample preparation

***Critical step for good quality RNA!



Sampling bias

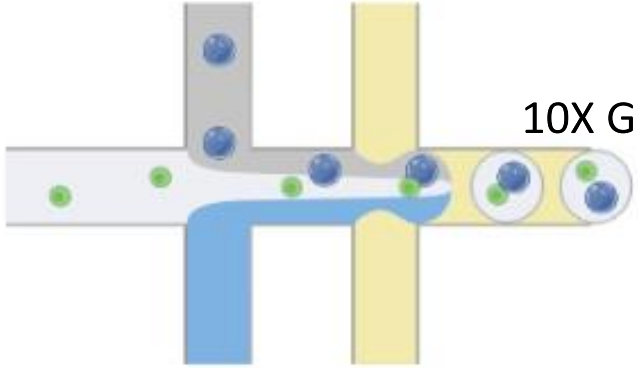
Bias from dissociation
: Enzymatic treatment, heat,
cell vs. nuclei

Steps of performing single cell RNA-seq
Cell capture and barcoding

② Cell capture and barcoding

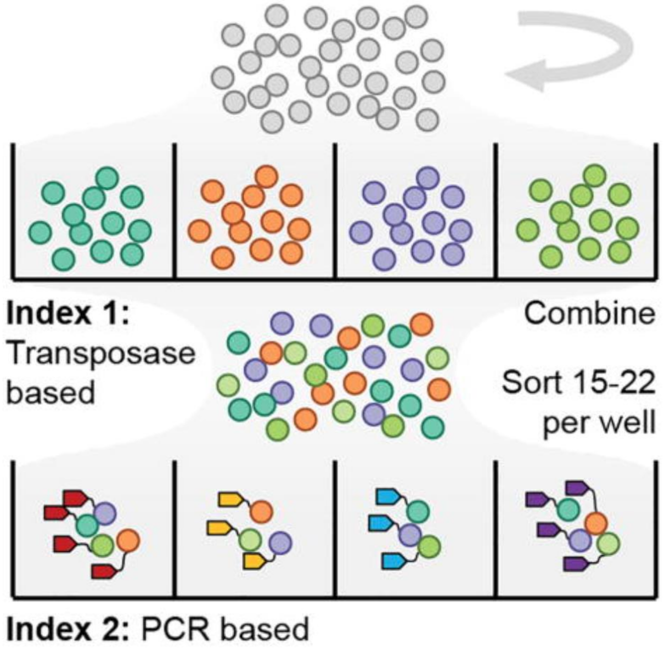
Cell per well

Smart-seq2 &3
CEL-seq
Fludigm C1
MARS-seq
SCRB-seq



Droplet

10X Genomics Chromium
Drop-seq
inDrops



Combinatorial index

Sci-seq

Vitak et al. Nature Methods 2017

Steps of performing single cell RNA-seq
Library Prep & Sequencing

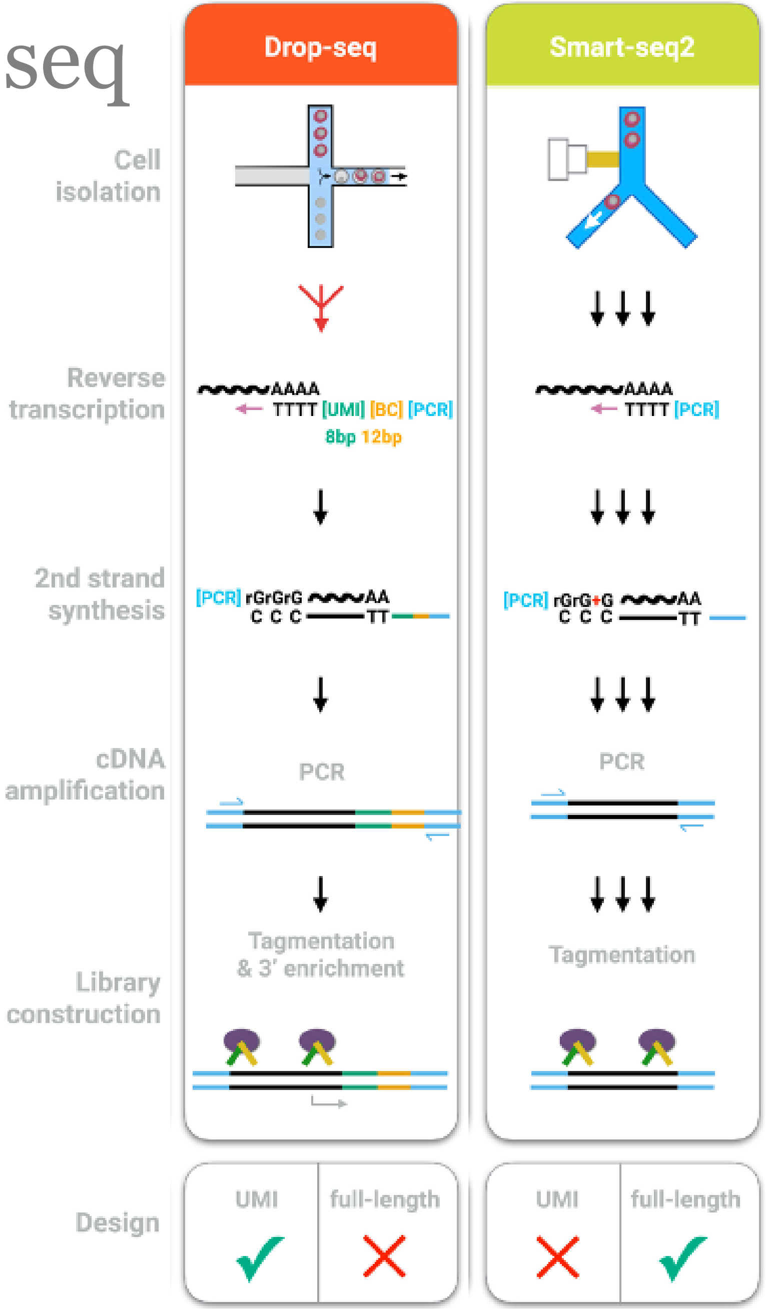
③ Library Prep

full -length vs.
3'/5' end tagging

PCR vs. in vitro
transcription

④ Single-cell sequencing

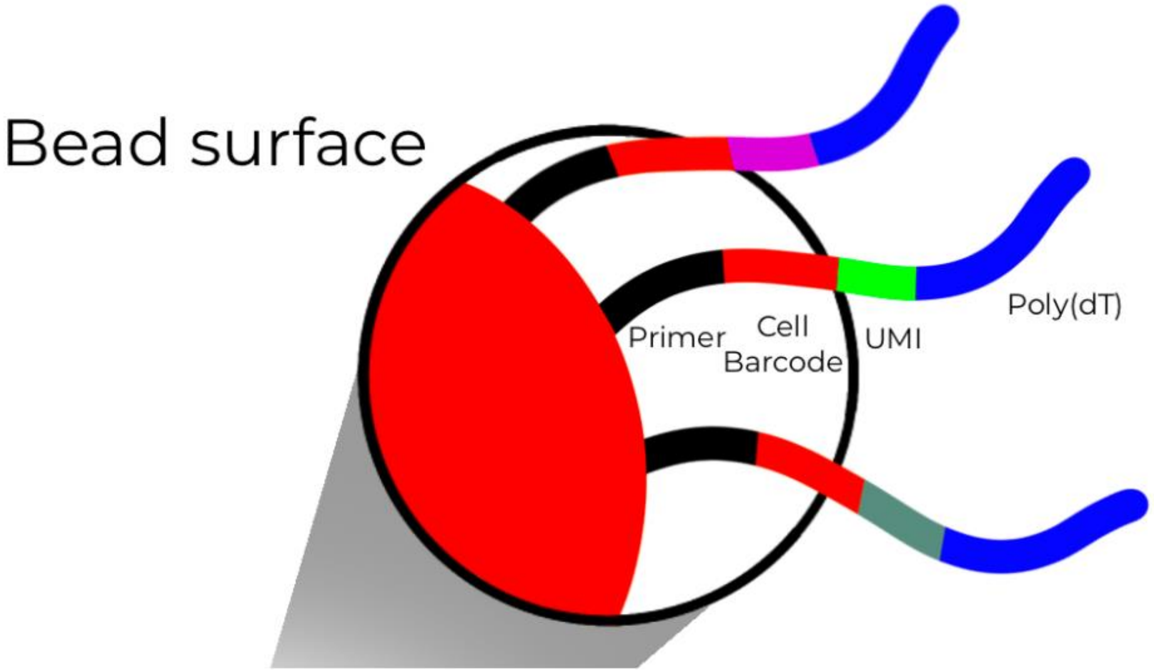
50,000 reads per cell



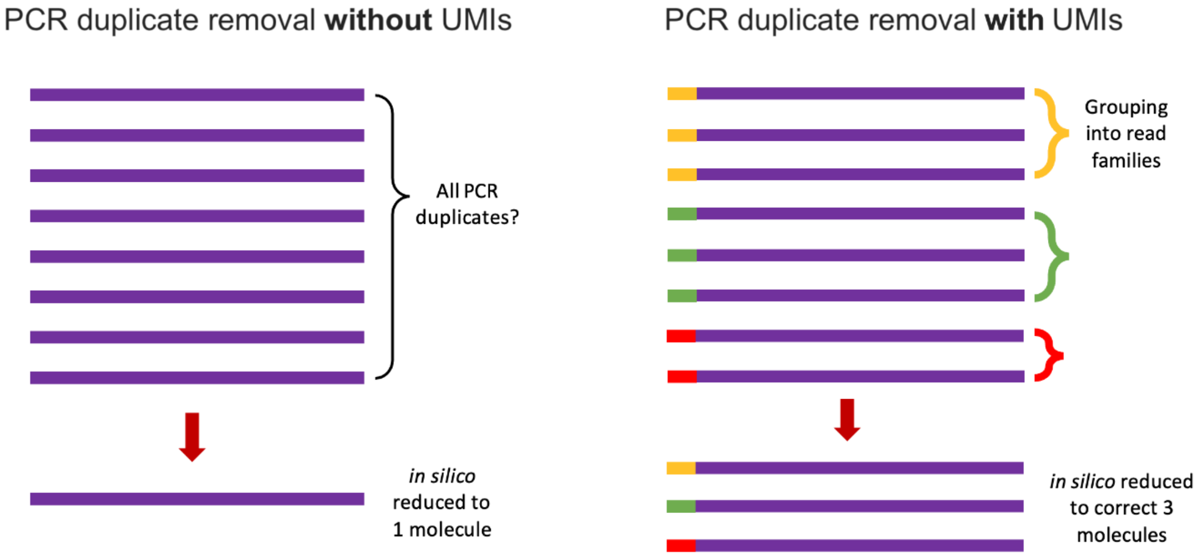
Ziegenhein et al. Molecular Cell 2017

Steps of performing single cell RNA-seq

Barcodes and UMIs



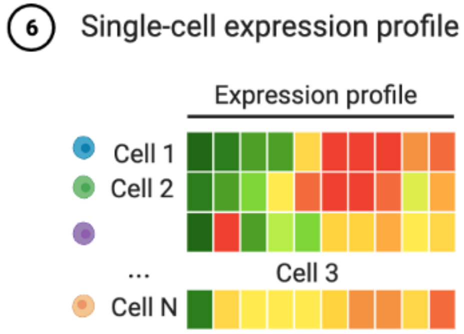
Each mRNA with unique UMI

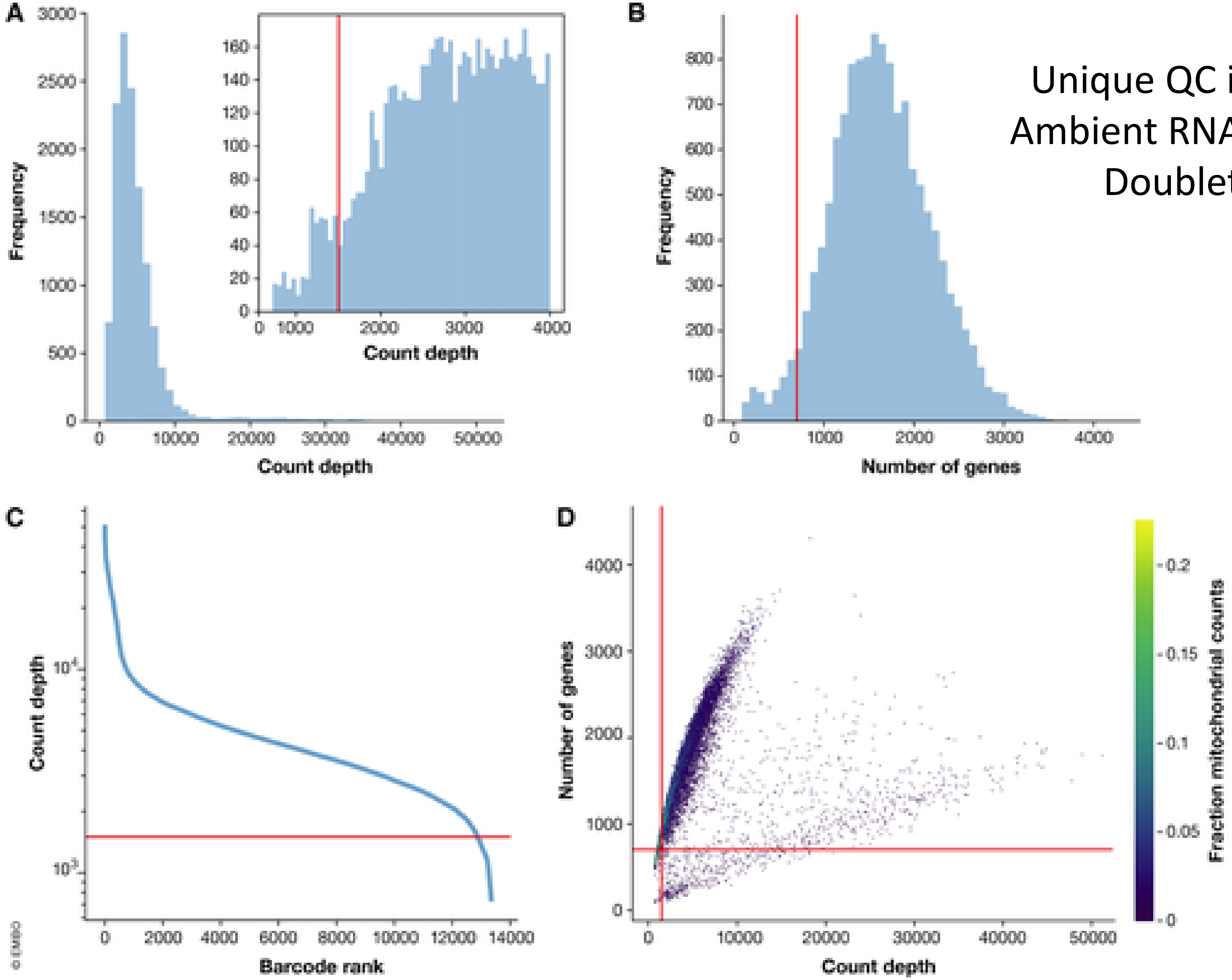


https://help.geneiousbiologics.com/hc/en-us/articles/4781289585300-Understanding-Single-Cell-technologies-Barcodes-and-UMIs

Steps of performing single cell RNA-seq
Preprocessing

FastQC
TrimPolyA
Alignment
BAM
Normalization
Batch correction





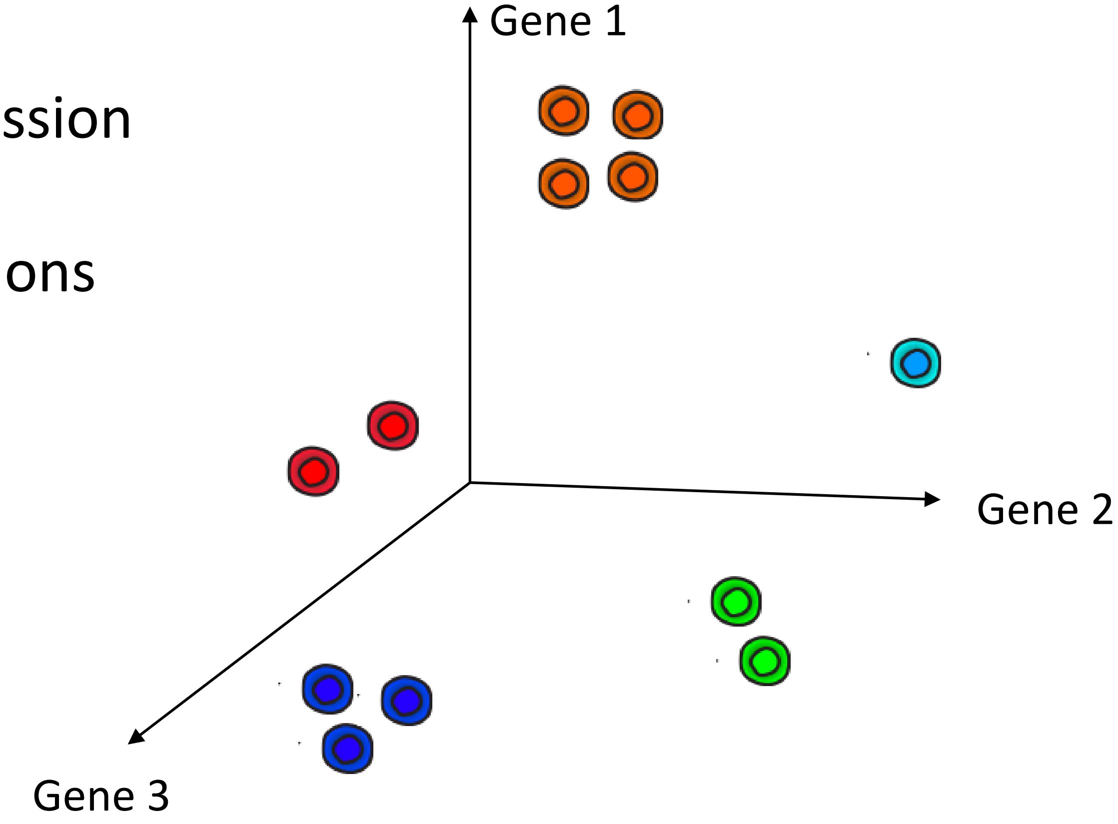
Unique QC issues:
Ambient RNA (soup)
Doublets

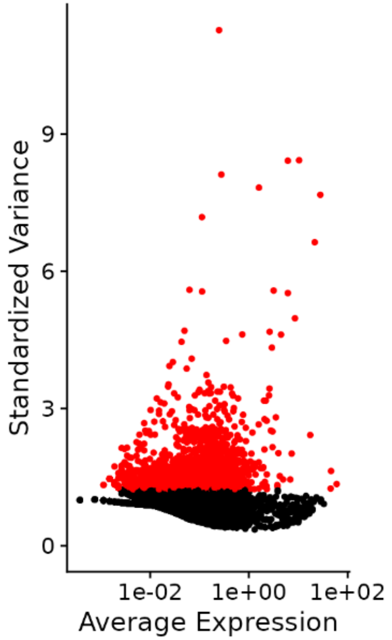
Luecken & Theis, Molecular systems biology 2019

Unique challenges of single-cell RNA sequencing

- Ultra-low input RNA
- Stochastic gene expression
- Sparse data
- Thousands of dimensions

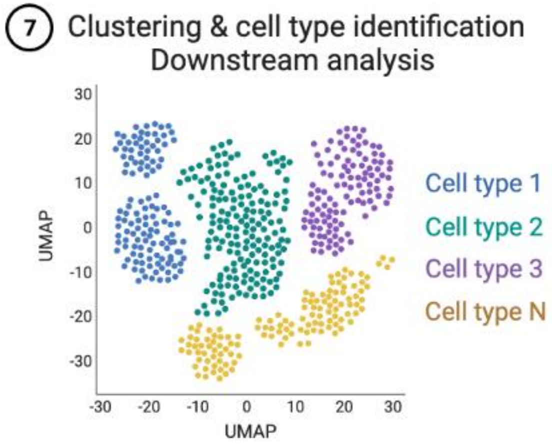
→ Feature selection





https://satijalab.org/seurat/articles/pbmc3k_tutorial.html

Steps of performing single cell RNA-seq
Downstream analysis: Visualization



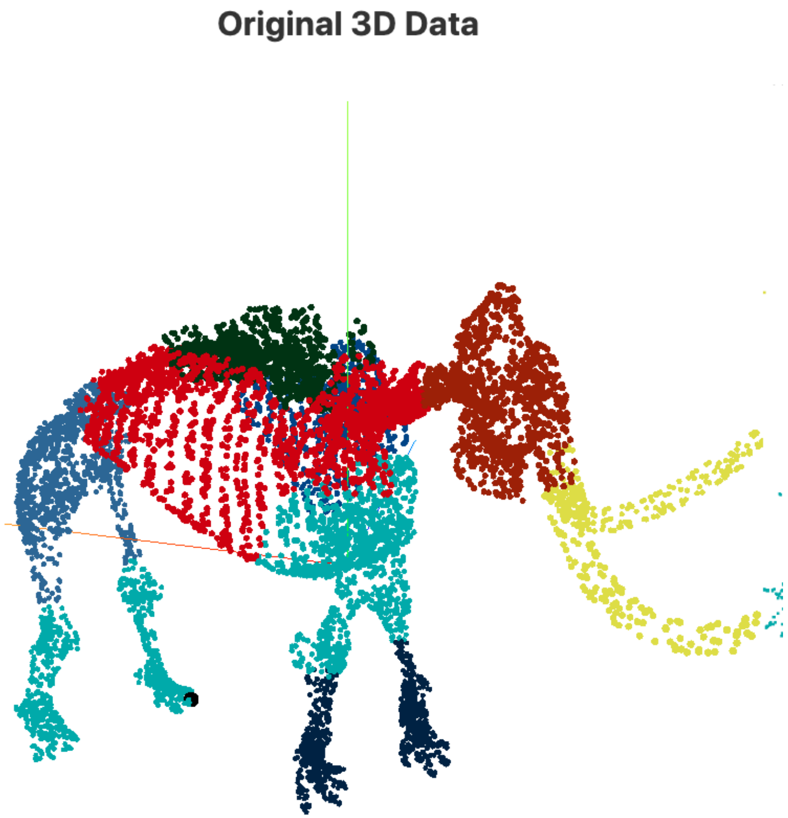
~20,000 dimensions

↓

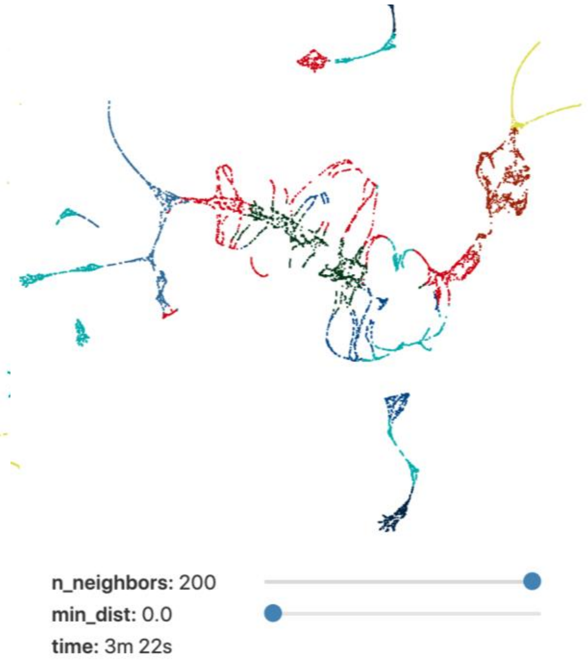
~20-50 dimension (PCA)

↓

2 dimensions



perplexity: 2000
time: 2h 5m

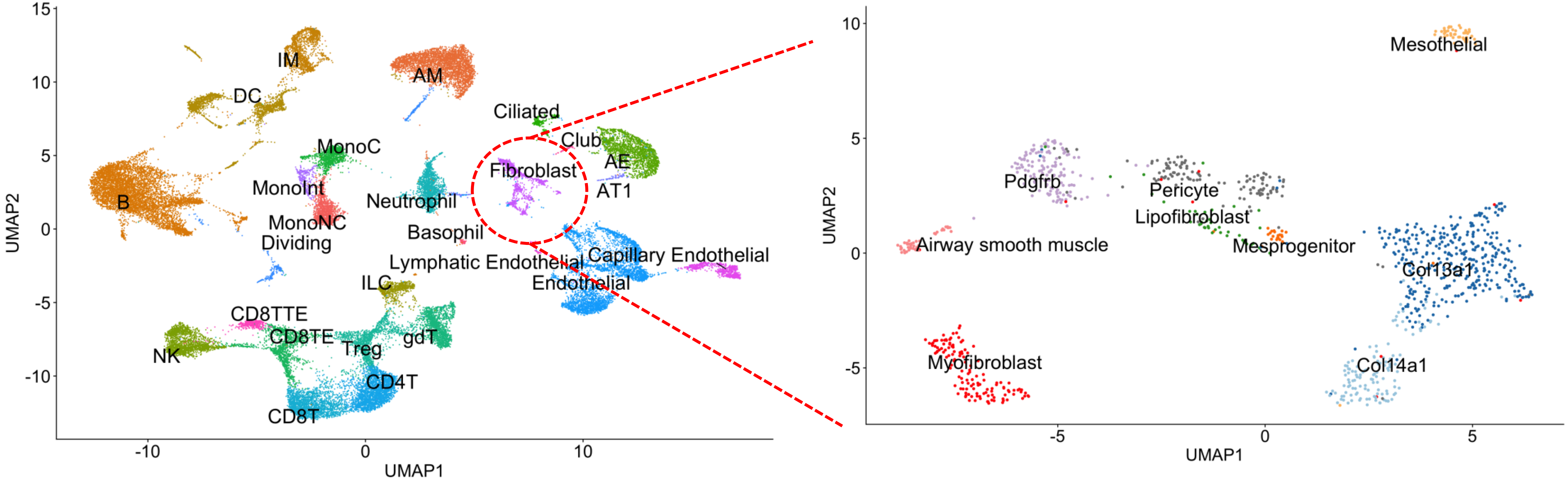


n_neighbors: 200
min_dist: 0.0
time: 3m 22s

https://pair-code.github.io/understanding-umap/

Steps of performing single cell RNA-seq
Downstream analysis: Clustering & marker identification

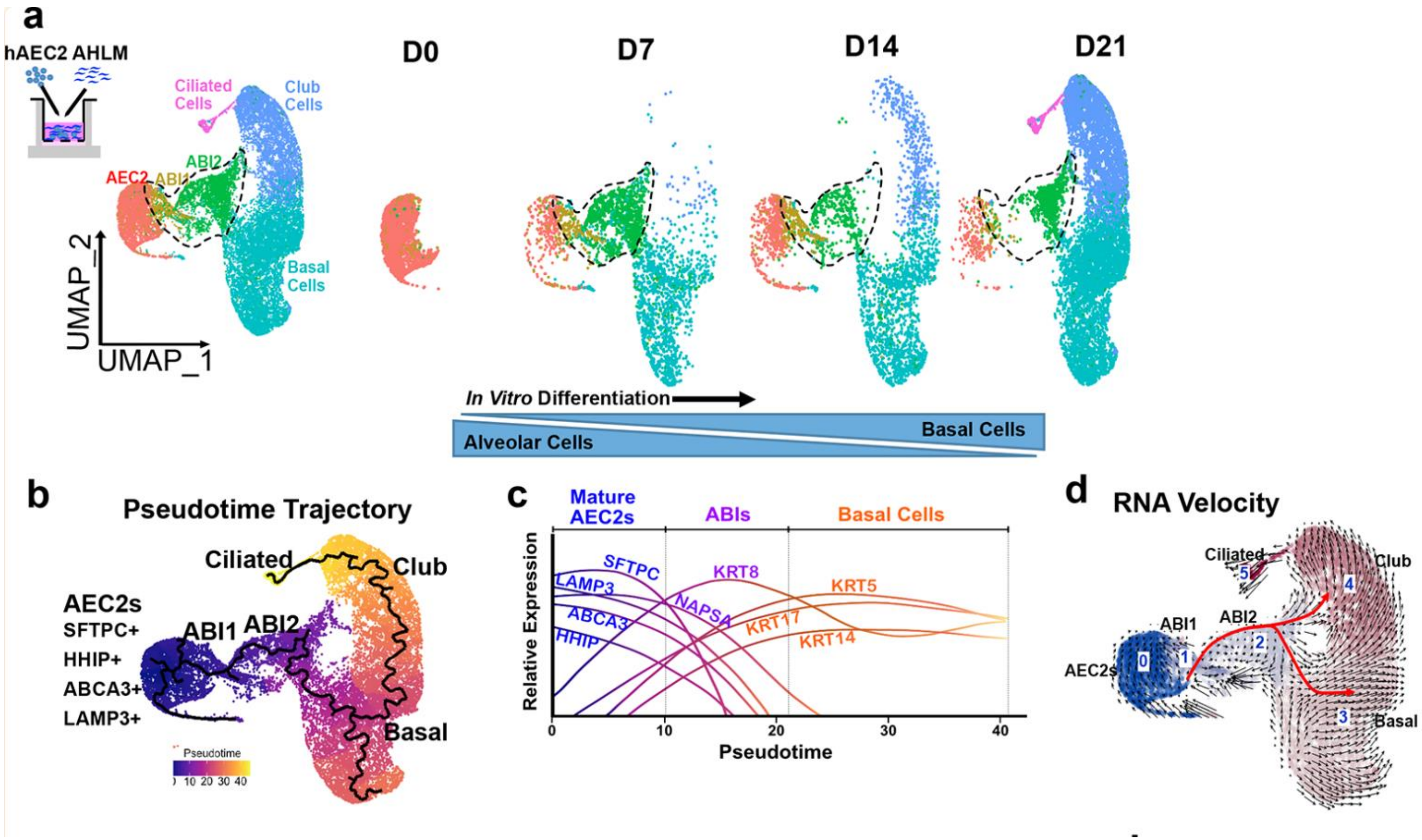
- Multiple different methods: hierarchical, graph based, k means etc
- Different methods give different results
- Test stability
- There is no gold standard answer



Yun et al. JCI Insight 2021

Steps of performing single cell RNA-seq
Downstream analysis: Others

- Differential expression
- Pathway analysis
- Interactome analysis
- Trajectory analysis
- Gene regulatory networks
- RNA velocity



Kathiryia & Wang et al. Nat Cell Biol. 2022

Best practices for single cell RNAseq experiment

실험 설계와 고려할 점

Considerations for single cell RNA-seq experiment
Protocols & Design

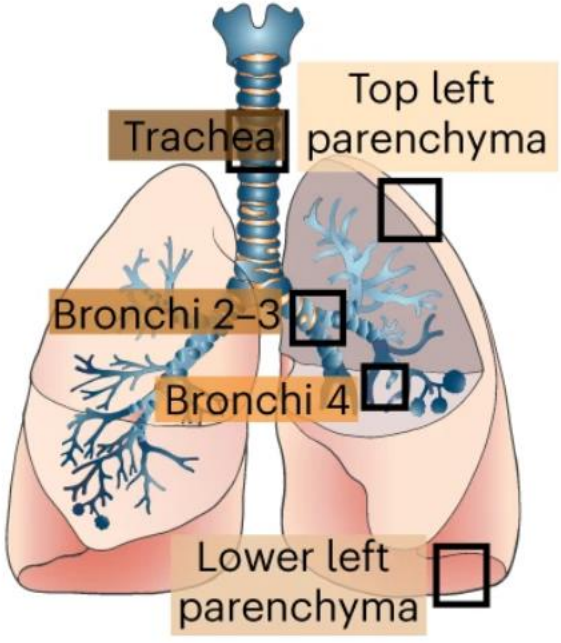
- 실험 설계에 고려할 점
  - 세포의 개수
  - 샘플을 얻는 방법
  - 예산과 시간
  - 유전자의 개수
  - Gene vs. isoform level

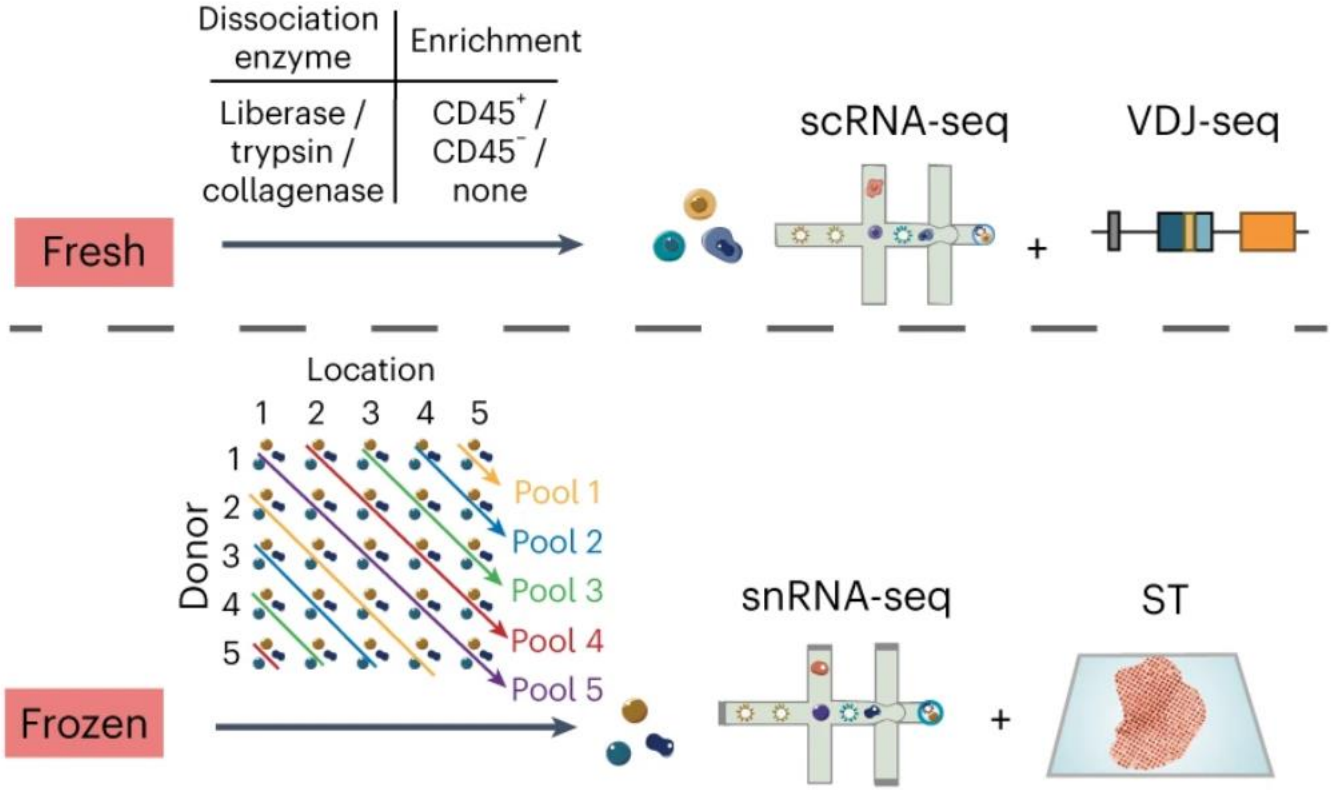
- **실험 방법과 설계는 실험 목적에 따라 결정한다.**
  - 폐조직의 다양한 세포를 알고 싶다
  - 질병군과 대조군의 차이를 알고 싶다
  - 과거에 보관해 놓은 환자 조직으로 연구를 하고 싶다
  - 기관지 내시경에서 얻은 세포의 구성을 알고 싶다
  - 암세포와 면역세포의 상호작용을 알고 싶다

Considerations for single cell RNA-seq experiment

Best practice

- 실험방법에 따른 편향을 검증할 계획을 실험 전에 세운다.





Travaglini et al. Nature 2020
Madisoon et al. Nature Genetics 2022

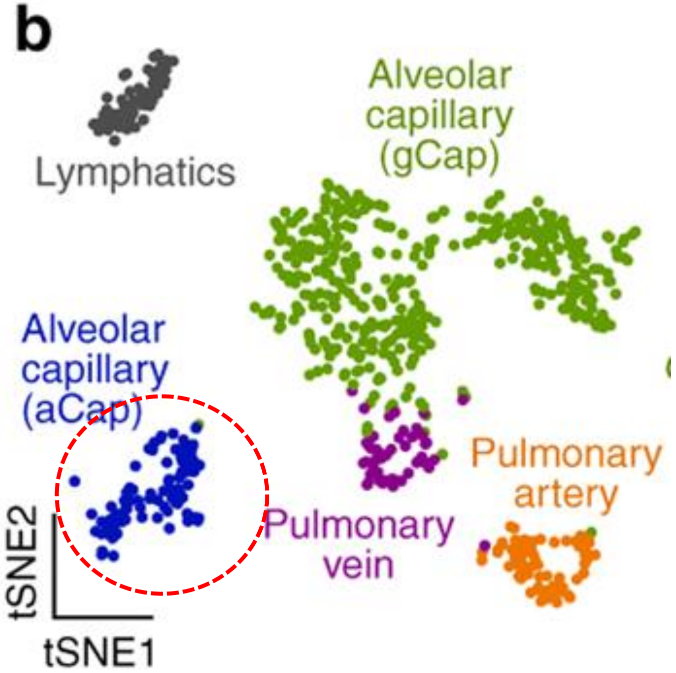
Considerations for single cell RNA-seq experiment
Analysis

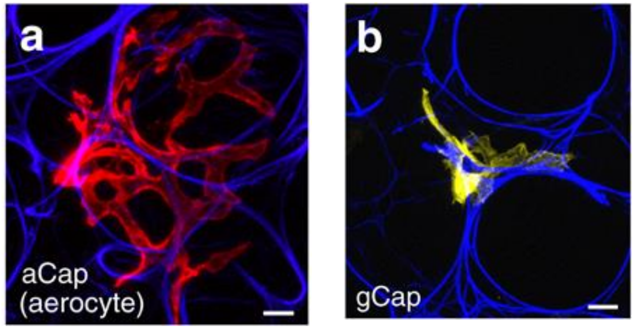
- **분석 방법은 매우 많고 빠르게 발전하므로, 실험 설계 당시와 다른 경우가 많다.**
- **분석 방법이 실험의 설계와 가정에 적합한지 확인한다.**
  - Seurat
  - SCANPY
  - Reference list 참조

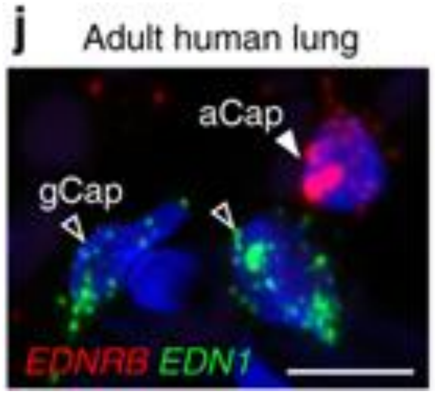
Single cell RNA-seq in lung biology and disease

Normal Lung
:Discovery of new cell types

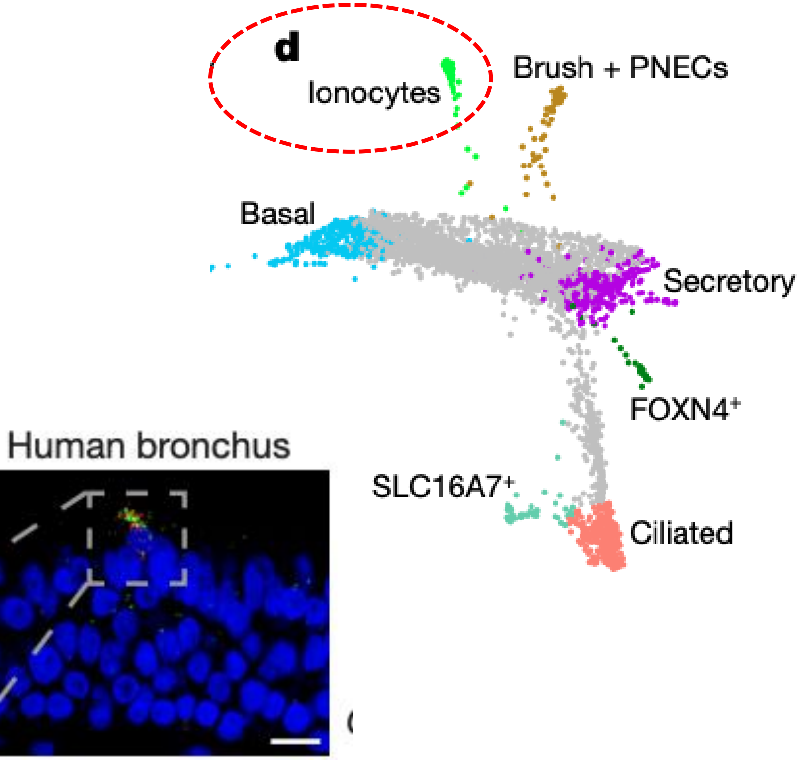
Aerocyte





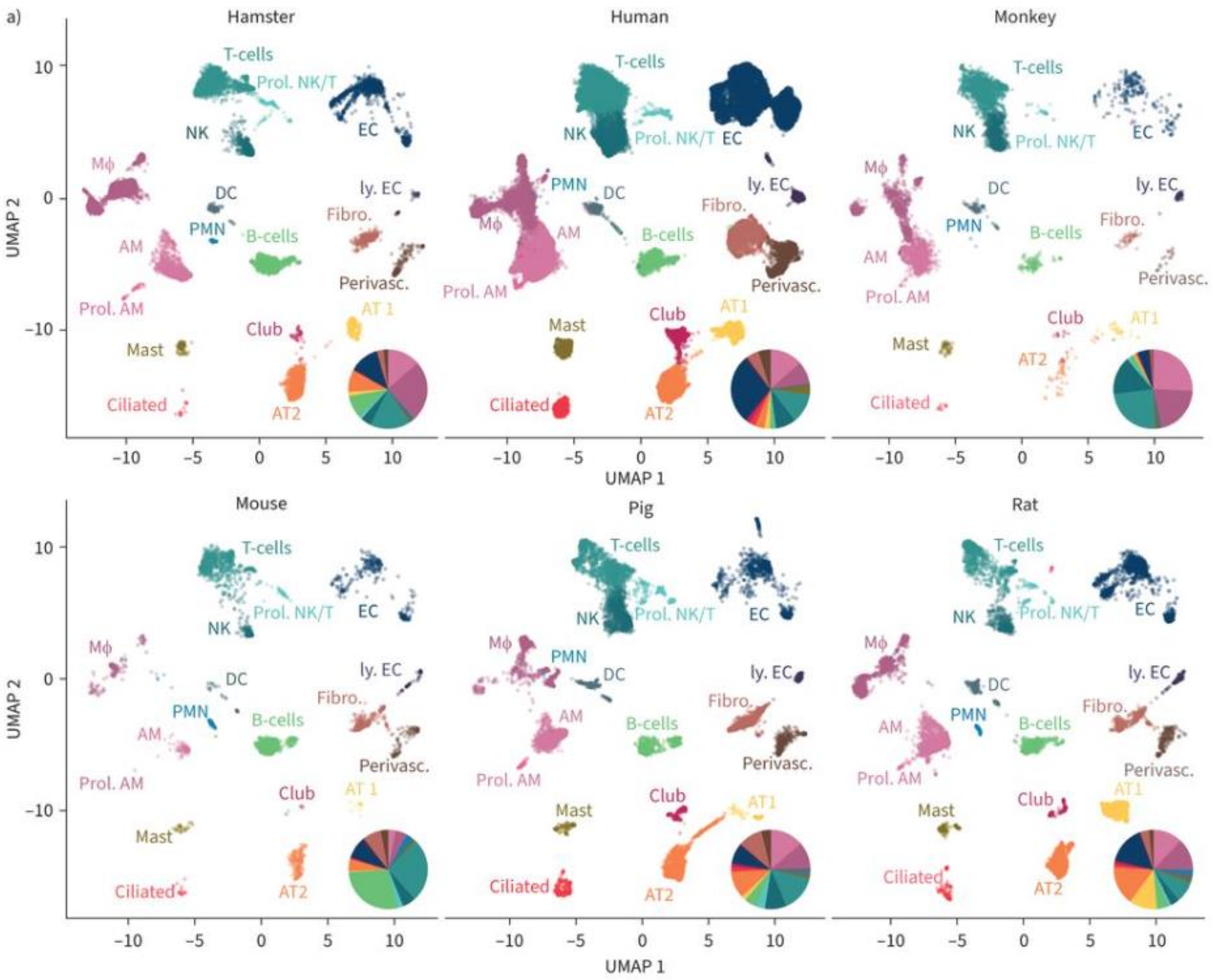


Ionocyte



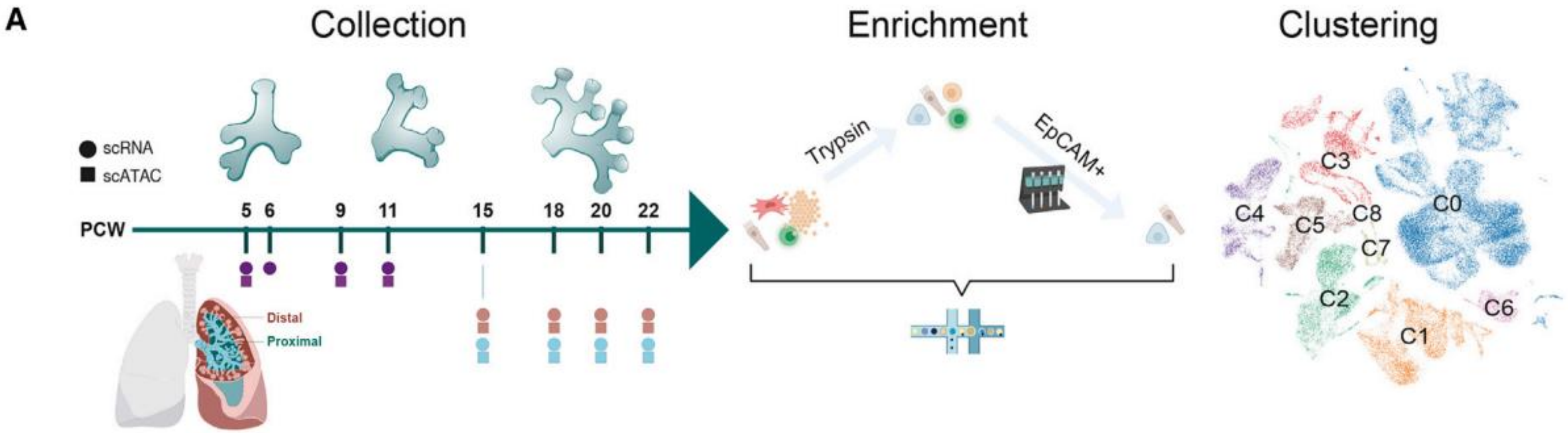
Plasschaert et al. Nature 2018
Gilllich et al. Nature 2020

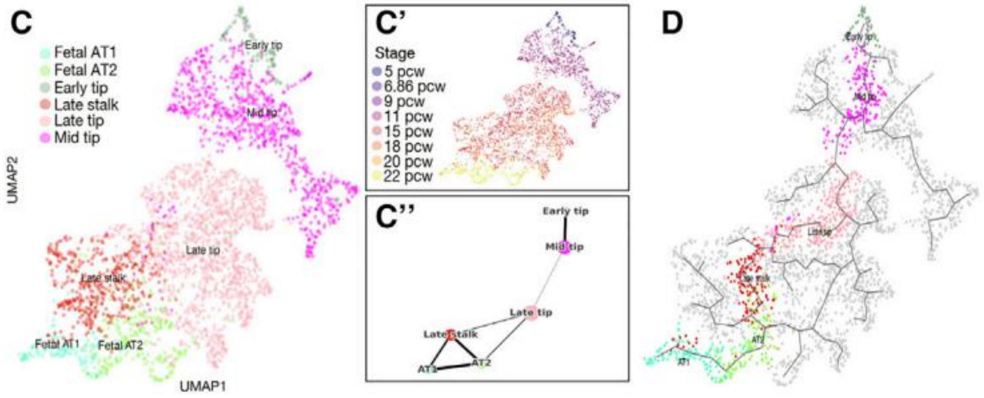
Normal Lung
: Cross-species comparison

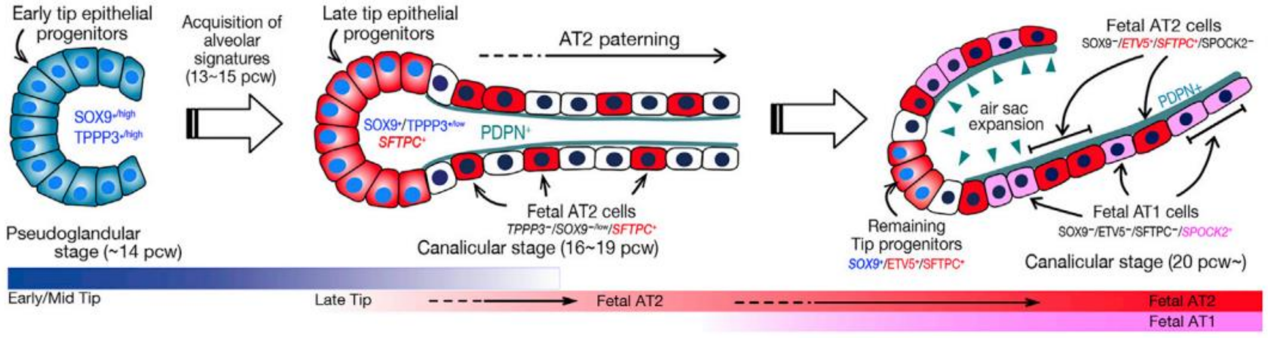


Pennitz et al. European Respiratory Review 2022

Normal Lung
: Developmental trajectories





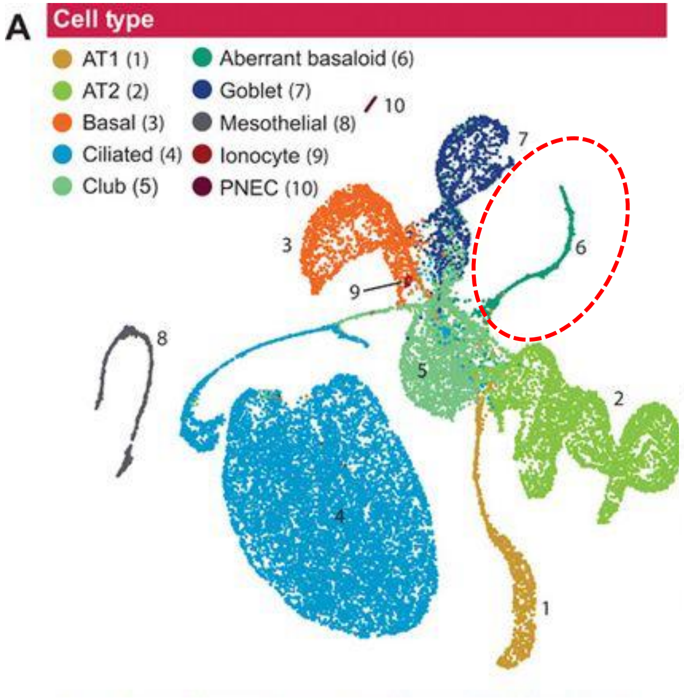


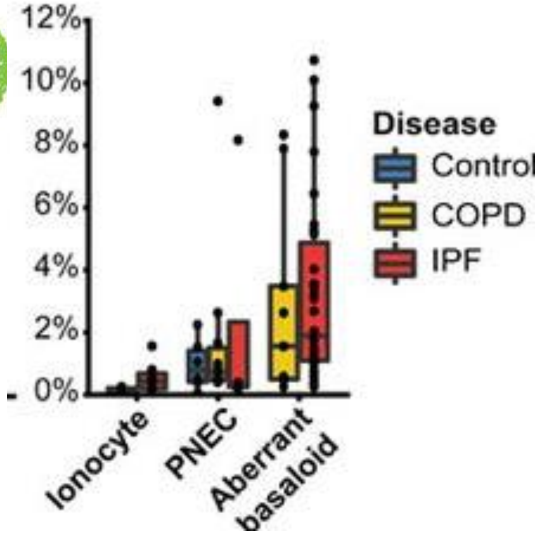
He et al. Cell 2022

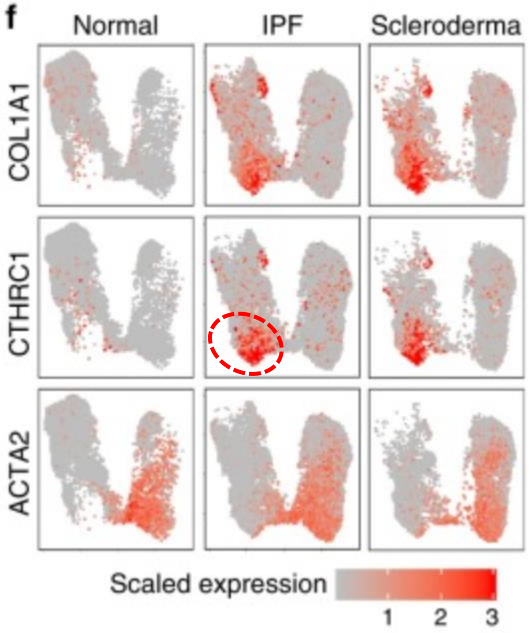
Idiopathic pulmonary fibrosis
: Altered cell populations

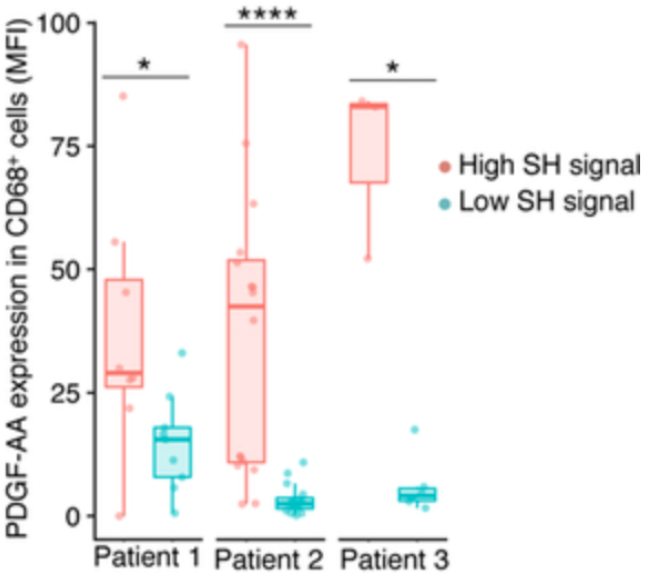
Aberrant Basaloid cells

Pro-fibrotic fibroblasts and macrophages



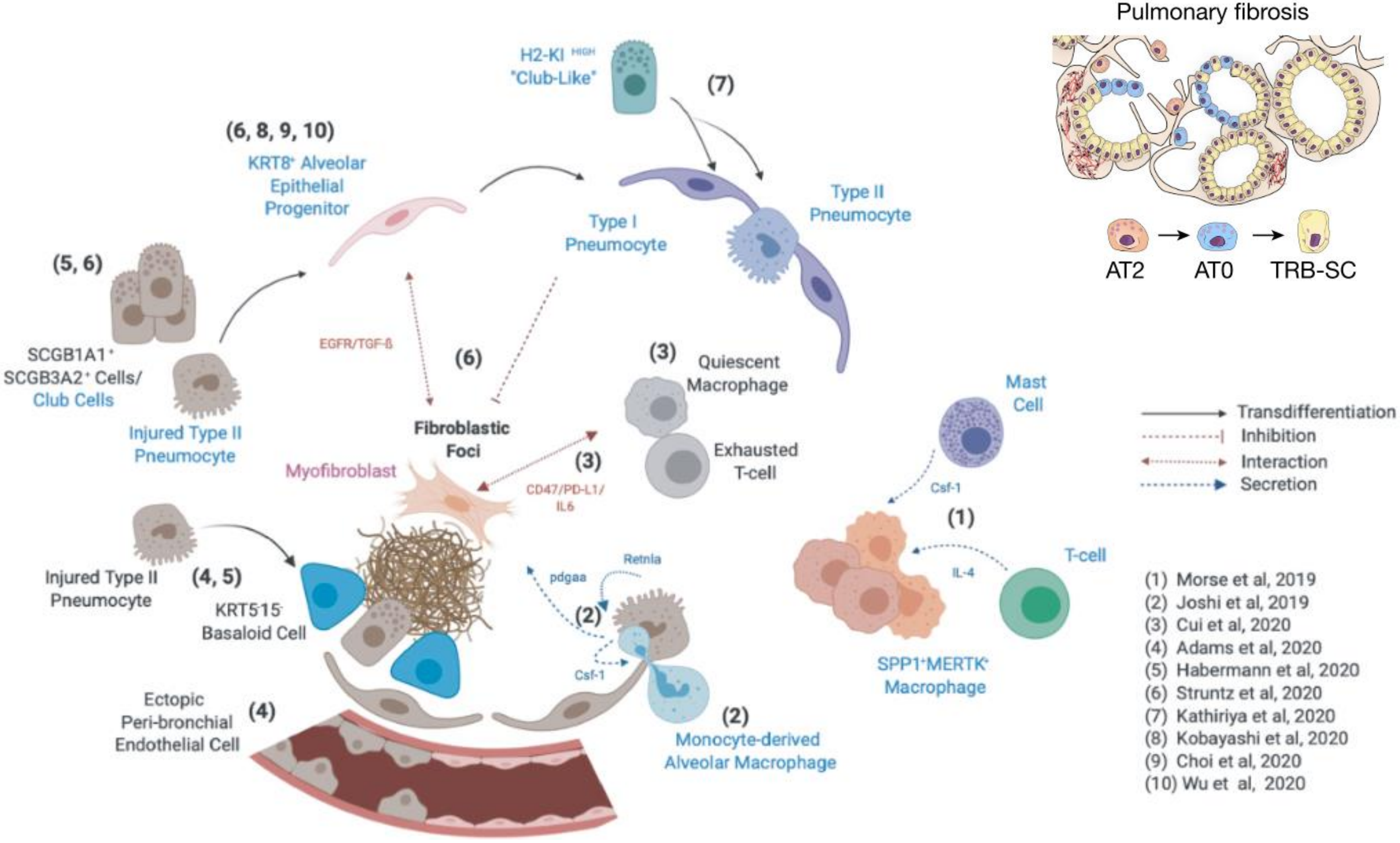






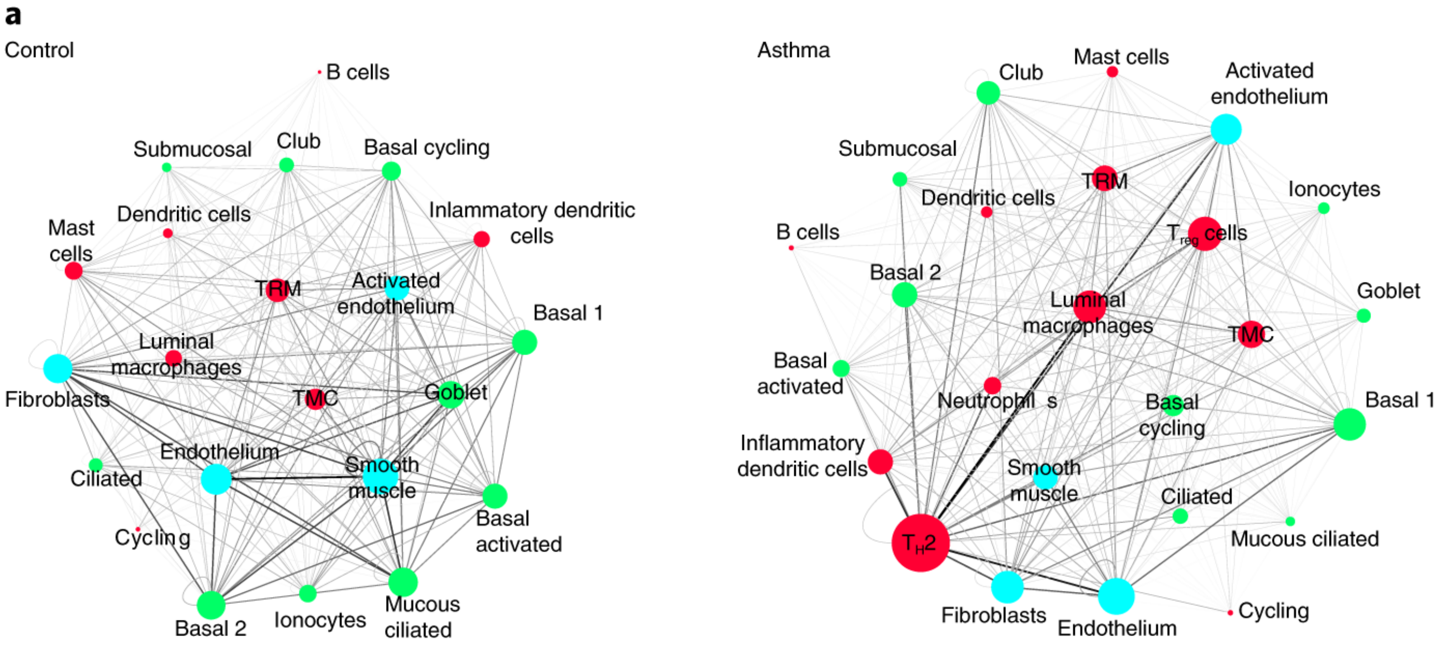
Adams et al. Science advances 2020
Tsukui et al. Nature Communications 2020
Aran et al. Nature Immunol.2019

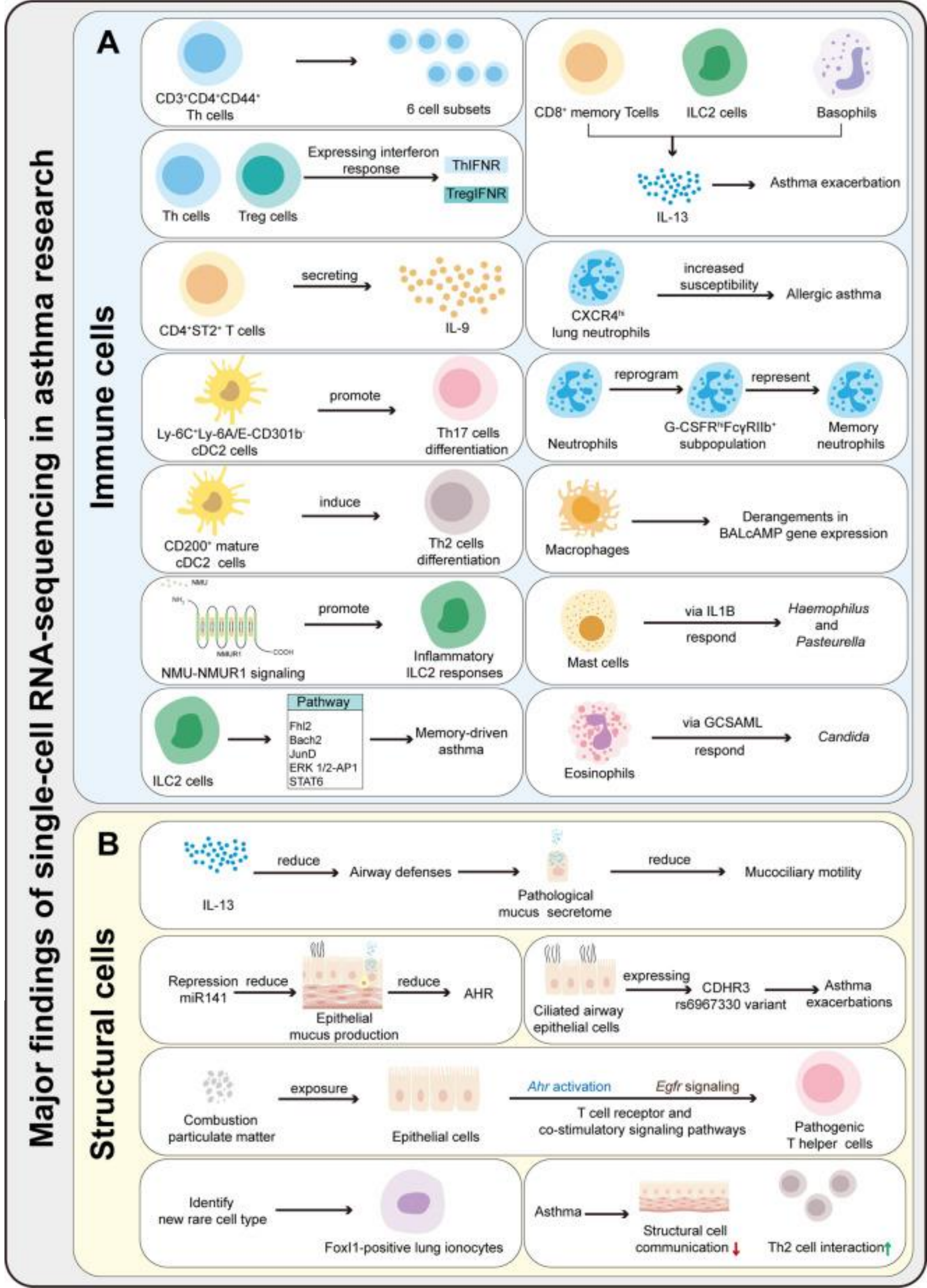
Idiopathic pulmonary fibrosis
: Altered cell transitions



Huang & Petretto AJP Lung 2021

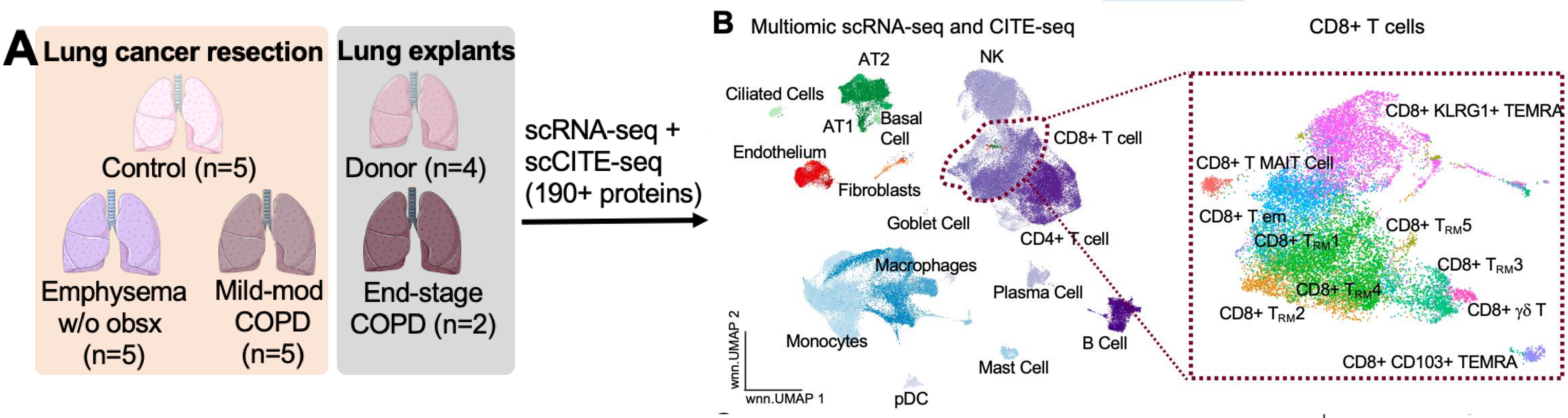
Asthma
: Altered cell-cell communications



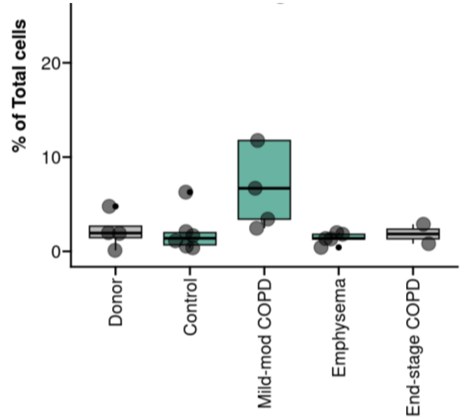


Barga et al. Nature Medicine 2019
Tang et al. Font. Immunol. 2022

Chronic Obstructive Pulmonary Disease
: Altered cell compositions



CD8+ KLRG1+ TEMRA



Vilasaenor Altamirano et al. (unpublished)

Limitations & Future Directions

단일세포 전사체 분석으로 알 수 없는 것
: We need to know what we don’t know

Bulk RNA seq

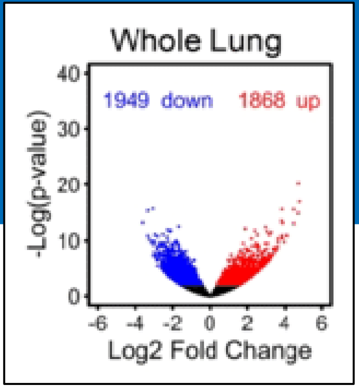
Single cell RNA seq

Reality
-Spatial organization
-Non-genetic signaling
-Long range interactions
-Microenvironment

단일세포 전사체 분석으로 알 수 없는 것
:Positional information

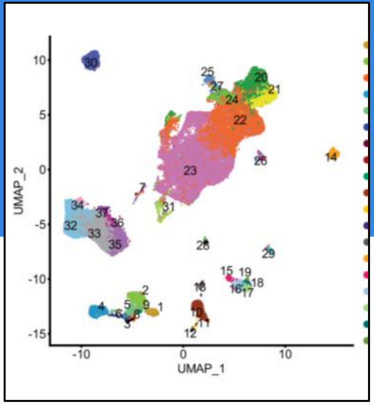
Bulk

- B cell signature



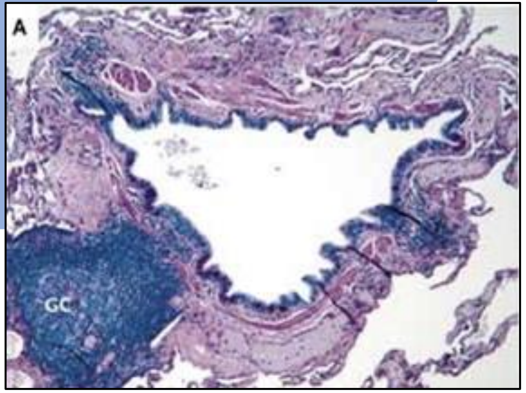
Single cell

- Lymphocyte clusters



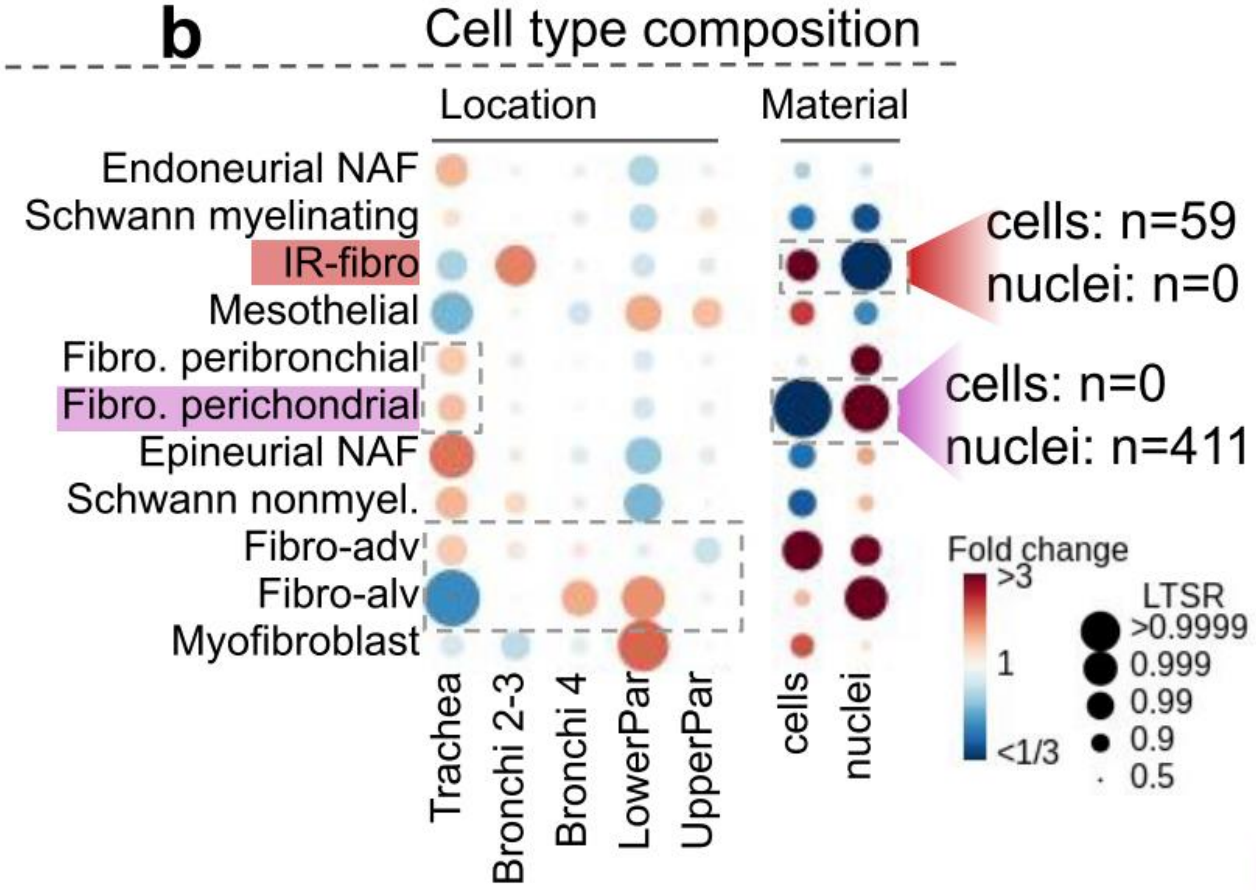
Spatial

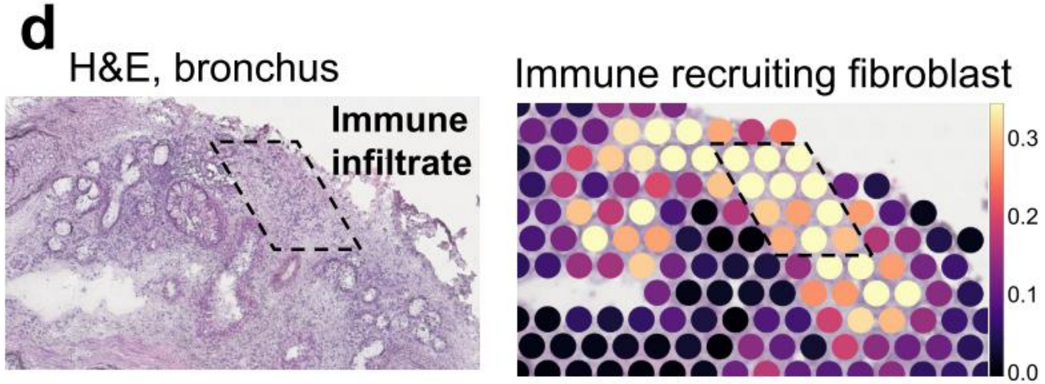
- Positional information



Hogg et al. NEJM 2004

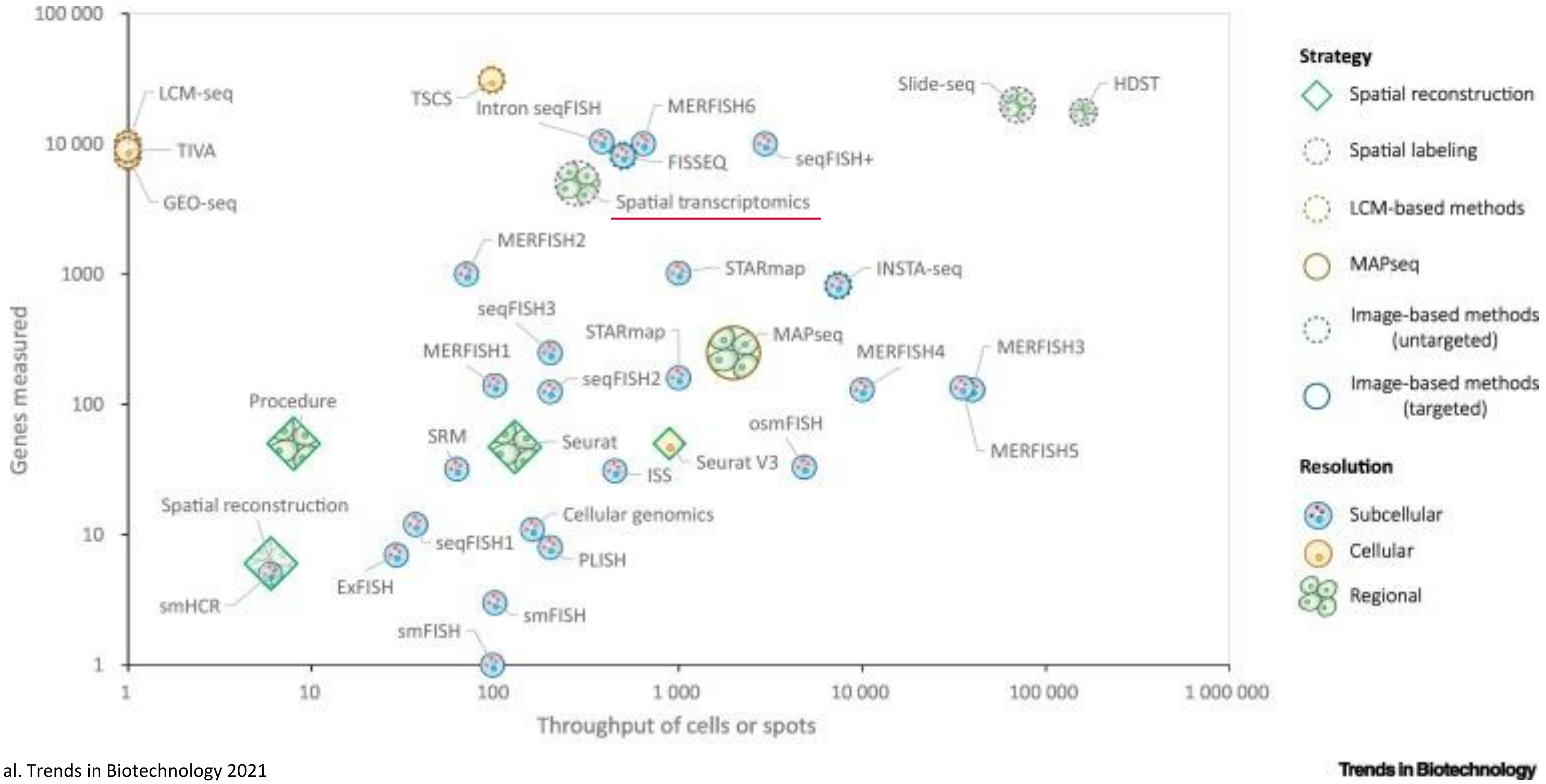
Combined single cell RNA seq and spatial transcriptomics identify novel fibroblast populations





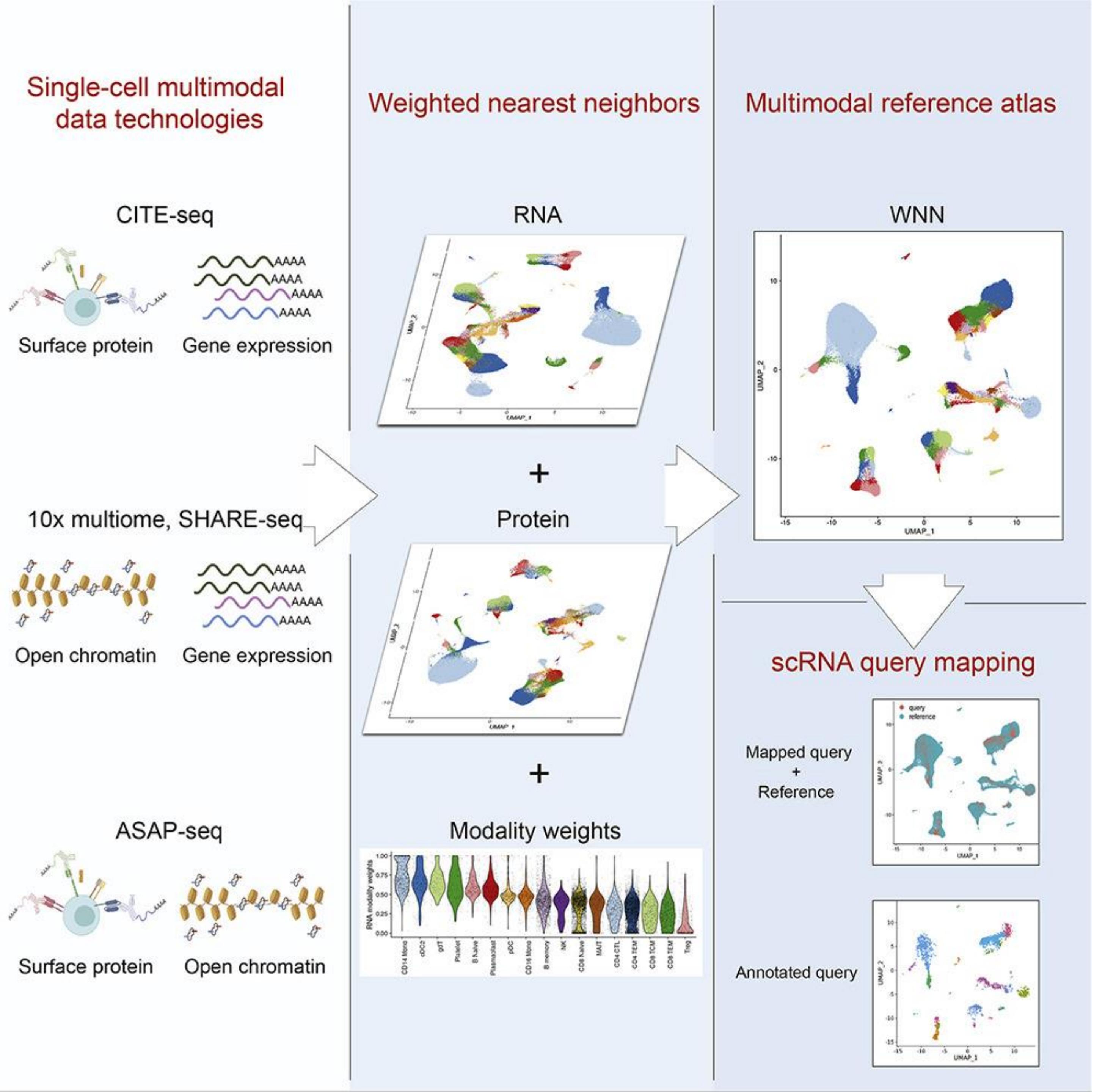
Madissoon et al. Nature Genetics 2022

Future Directions
: Spatial Genomics



Liao et al. Trends in Biotechnology 2021

Future Directions
: Single cell multi-omics

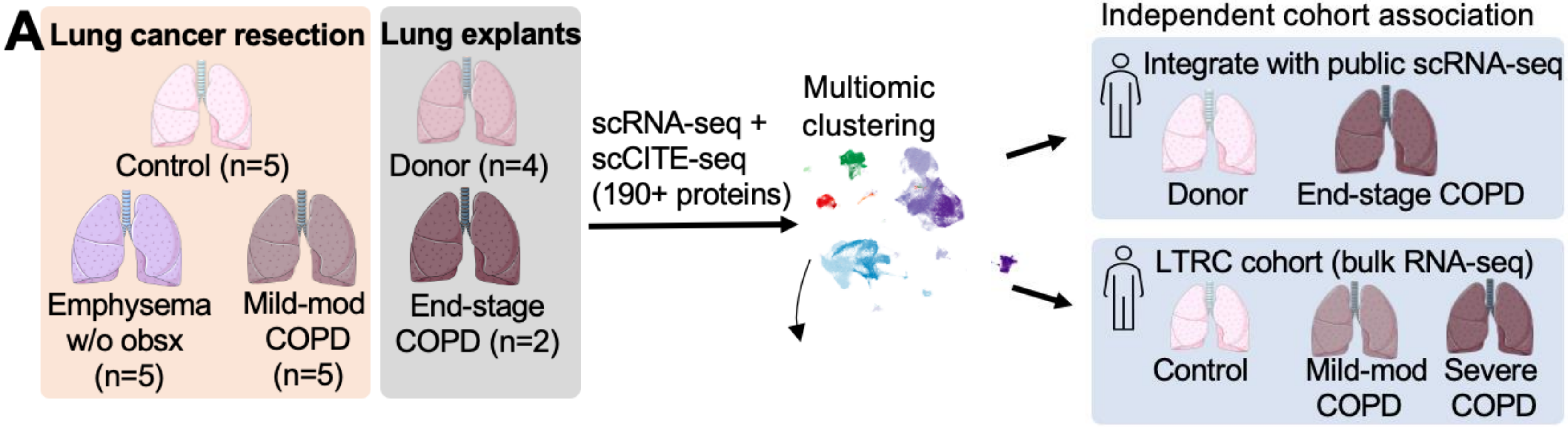


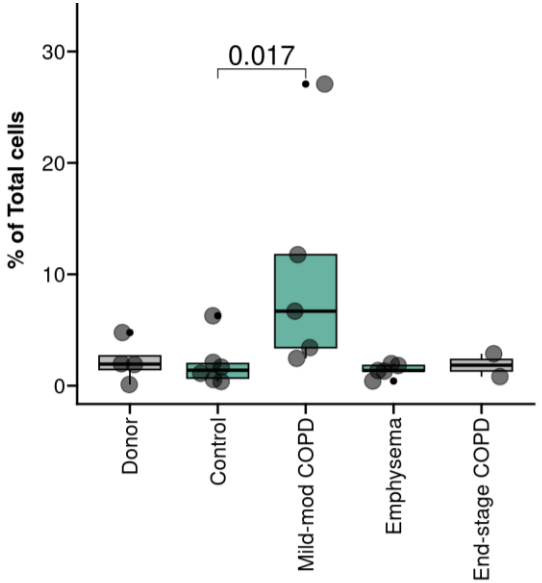
Hao et al. Cell 2021

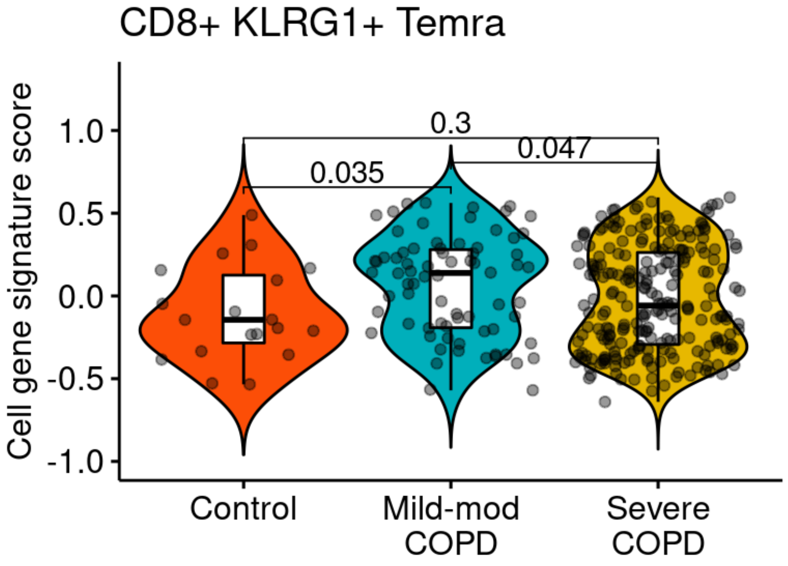
단일세포 전사체 분석으로 알 수 없는 것
: population level diversity

- Disease heterogeneity
- Difficult to access tissue
- Experimental bias
- Cost

Future Directions
:Deconvolution of bulk RNA seq

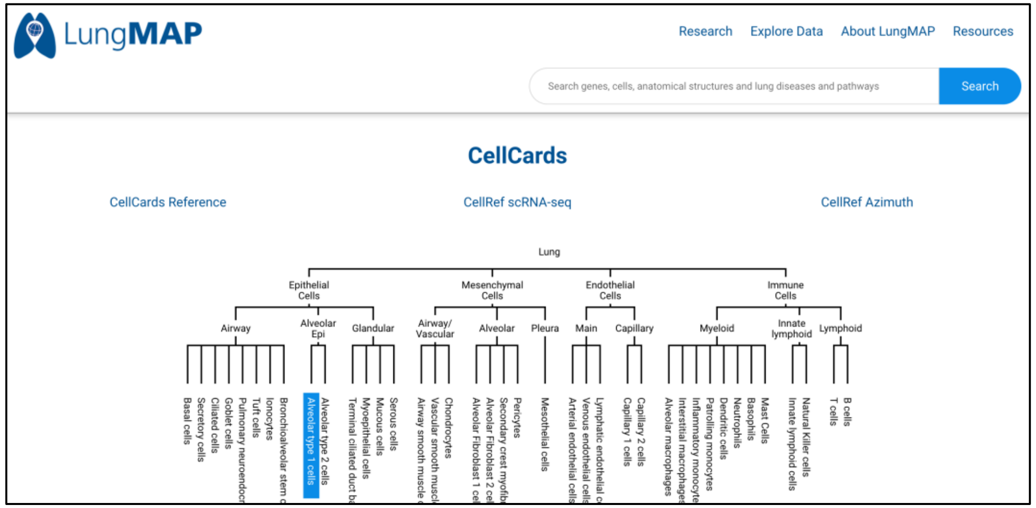


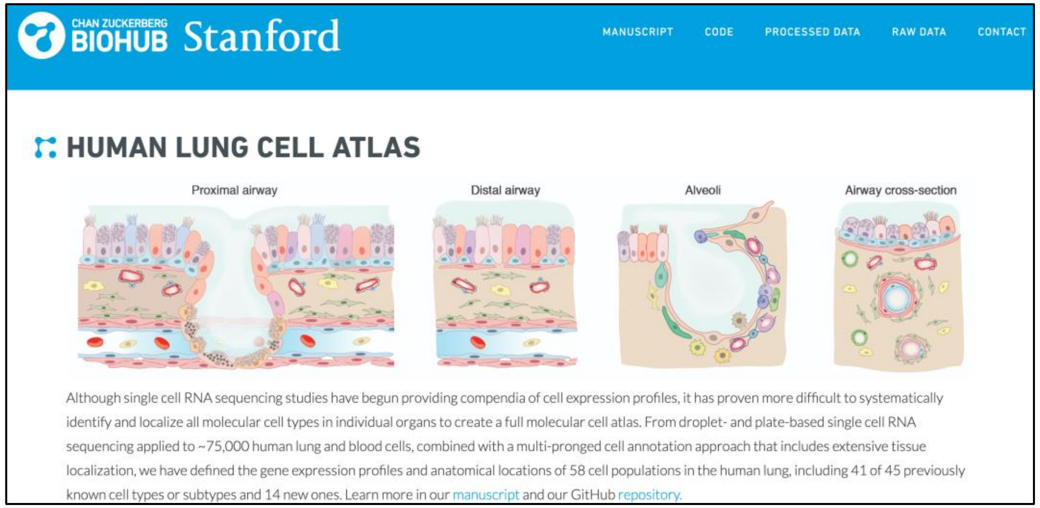




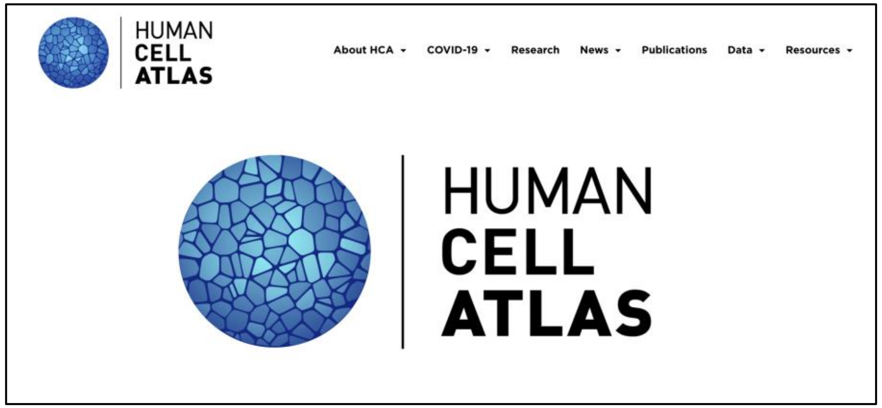
Vilasaenor Altamirano et al. (unpublished)

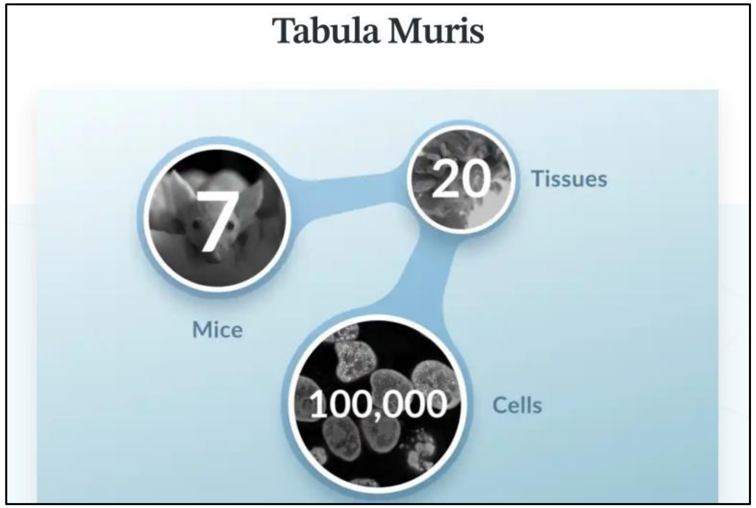
Putting it altogether
:Comprehensive atlas of cells





Although single cell RNA sequencing studies have begun providing compendia of cell expression profiles, it has proven more difficult to systematically identify and localize all molecular cell types in individual organs to create a full molecular cell atlas. From droplet- and plate-based single cell RNA sequencing applied to ~75,000 human lung and blood cells, combined with a multi-pronged cell annotation approach that includes extensive tissue localization, we have defined the gene expression profiles and anatomical locations of 58 cell populations in the human lung, including 41 of 45 previously known cell types or subtypes and 14 new ones. Learn more in our manuscript and our GitHub repository.





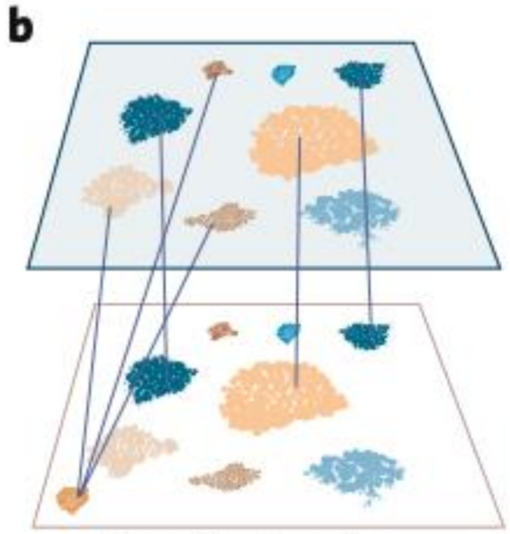
https://www.lungmap.net/cell-cards/
https://hlca.ds.czbiohub.org/
https://www.czbiohub.org/sf/tab
https://www.humancellatlas.org/
https://www.czbiohub.org/sf/tabula-muris/

Future Directions
:Data integration, access and query

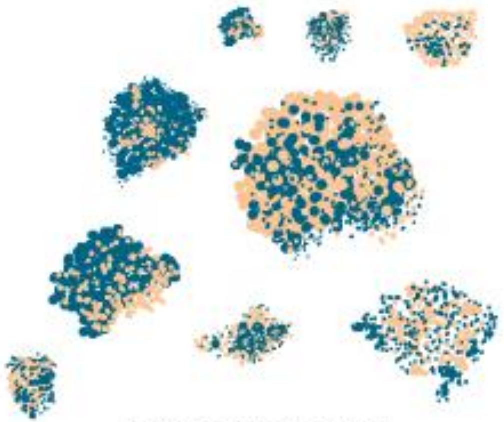
Single cell RNA seq
RNA seq
Single cell ATAC seq
Imaging
Proteomics
Spatial genomics
…

Meta analysis

New data
Query similar cell types
Different organisms or diseases
Cell types with similar genes



Transfer learning



Joint clustering

Efremova & Teichmann Nature Methods 2020

In closing

- Single cell RNA sequencing has become robust and broadly accessible, now with millions of cells
- Single cell RNA sequencing enables identification of biologically relevant heterogeneity between cells.
- The influence of single cell RNA seq on respiratory research will grow as the experimental and computational methods improve for the multi-omic integration.

Alexander et al. European Respiratory Review 2020

감사합니다

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References

Adams et al. Science Advances 2020
Aibar et al. Nature Methods 2017
Alexander et al. European Respiratory Review 2020
Aran et al. Nature Immunology 2019
Armingo et al. Nature Reviews Genetics 2021
Basil et al. Nature 2022
Carraro et al. Journal of Pathology 2022
Delorey et al. Nature 2021
Habermann et al. Science Advances 2020
Hao et al. Cell 2021
He et al. Cell 2022
Kadur Lakshminarasimha Murthy et al. Nature 2022
Kiselev et al. Nature Reviews Genetics 2019
Leucken & Theis, Molecular Systems Biology 2019
Madissoon et al. Nature Genetics 2022
Pour et al. Developmental Cell 2022
Stewart et al. nature Reviews Genetics 2019
Svensson et al. Nature Methods 2017
Travaglini et al. Nature 2020
Tsukui et al. Nature Communications 2020
Wang et al. eLife 2020
You et al. Genome Biology 2021
https://github.com/seandavi/awesome-single-cell
https://satijalab.org/seurat/
https://www.scrna-tools.org/
https://www.singlecellcourse.org/
http://www.ipfcellatlas.com/
https://www.lungmap.net/
https://www.lungcellatlas.org/
https://hlca.ds.czbiohub.org/
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