KSBi-BIML 2024

Bioinformatics & Machine Learning(BIML) Workshop for Life and Medical Scientists

생명정보학 & 머신러닝 워크샵 (온라인)

Single-cell RNA-sequencing analysis of cancer

이세민 _ UNIST

KSBI KOREAN SOCIETY FOR BIOINFORMATICS | 한국생명정보학회

본 강의 자료는 한국생명정보학회가 주관하는 BIML 2024 워크샵 온라인 수업을 목적으로 제작된 것으로 해당 목적 이외의 다른 용도로 사용할 수 없음을 분명하게 알립니다.

이를 다른 사람과 공유하거나 복제, 배포, 전송할 수 없으며 만약 이러한 사항을 위반할 경우 발생하는 **모든 법적 책임은 전적으로 불법 행위자 본인에게 있음을 경고**합니다.

KSBi-BIML 2024

Bioinformatics & Machine Learning(BIML)
Workshop for Life and Medical Scientists

안녕하십니까?

한국생명정보학회가 개최하는 동계 교육 워크샵인 BIML-2024에 여러분을 초대합니다. 생명정보학 분야의 연구자들에게 최신 동향의 데이터 분석기술을 이론과 실습을 겸비해 전달하고자 도입한 전문 교육 프로그램인 BIML 워크샵은 2015년에 시작하여 올해로 벌써 10년 차를 맞이하게 되었습니다. BIML 워크샵은 국내 생명정보학 분야의 최초이자 최고 수준의 교육프로그램으로 크게 인공지능과 생명정보분석 두 개의 분야로 구성되어 있습니다. 올해 인공지능 분야에서는 최근 생명정보 분석에서도 응용이 확대되고 있는 다양한 인공지능 기반 자료모델링 기법들에 대한 현장 강의가 진행될 예정이며, 관련하여 심층학습을 이용한 단백질구조예측, 유전체분석, 신약개발에 대한 이론과 실습 강의가 함께 제공될 예정입니다. 또한 단일세포오믹스, 공간오믹스, 메타오믹스, 그리고 롱리드염기서열 자료 분석에 대한 현장 강의는 많은 연구자의 연구 수월성 확보에 큰 도움을 줄 것으로 기대하고 있습니다.

올해 BIML의 가장 큰 변화는 최근 연구 수요가 급증하고 있는 의료정보자료 분석에 대한 현장 강의를 추가하였다는 것입니다. 특히 의료정보자료 분석을 많이 수행하시는 의과학자 및 의료정보 연구자들께서 본 강좌를 통해 많은 도움을 받으실 수 있기를 기대하고 있습니다. 또한 다양한 생명정보학 분야에 대한 온라인 강좌 프로그램도 점차 증가하고 있는 생명정보 분석기술의 다양화에 발맞추기 위해 작년과 비교해 5강좌 이상을 신규로 추가했습니다. 올해는 무료 강좌 5개를 포함하여 35개 이상의 온라인 강좌가 개설되어 제공되며, 연구 주제에 따른 연관된 강좌 추천 및 강연료 할인 프로그램도 제공되며, 온라인을 통한 Q&A 세션도 마련될 예정입니다. BIML-2024는 국내 주요 연구중심 대학의 전임 교원이자 각 분야 최고 전문가들의 강의로 구성되었기에 해당 분야의 기초부터 최신 연구 동향까지 포함하는 수준 높은 내용의 강의가 될 것이라 확신합니다.

BIML-2024을 준비하기까지 너무나 많은 수고를 해주신 운영위원회의 정성원, 우현구, 백대현, 김태민, 김준일, 김상우, 장혜식, 박종은 교수님과 KOBIC 이병욱 박사님께 커다란 감사를 드립니다. 마지막으로 부족한 시간에도 불구하고 강의 부탁을 흔쾌히 허락하시고 훌륭한 현장 강의와 온라인 강의를 준비하시는데 노고를 아끼지 않으신 모든 강사분들께 깊은 감사를 드립니다.

2024년 2월

한국생명정보학회장 이 인 석

강의개요

Single-cell RNA-sequencing analysis of cancer

본 강좌에서는 최근 각광 받고 있는 단세포 전사체 데이터 분석 기술에 대한 소개와 실제 데이터에 대한 분석 실습을 병행하고자 한다. 단세포 전사체 분석 기술은 세포의 분화, 암의 진화, 면역세포 프로파일링 및 종양 내 이질성 분석 등에 활용되고 있으며, 관련 기술과 응용 사례에 대한 소개 및 현재 가장 널리 사용되고 있는 10x Genomics사의 Chromium Single Cell Gene Expression Solution을 사용하여 생산된 암샘플 단세포 전사체 데이터를 위주로 다양한 분석 방법에 대한 실습을 진행하고자 한다. 강의는 다음의 내용을 포함한다.

- Single-cell RNA-sequencing(scRNA-seq)의 소개 및 개요
- 암 scRNA-seq 연구 동향
- 암 scRNA-seq 데이터 분석 실습

* 교육생준비물: 노트북 (메모리 8GB 이상, 디스크 여유공간 30GB 이상)

* 강의: 이세민 교수 (울산과학기술원 바이오메디컬공학과)
  실습: 정형오 박사 (울산과학기술원 바이오메디컬공학과)

Curriculum Vitae

Speaker Name: Semin Lee, Ph.D.

▶ **Personal Info**

Name	Semin Lee
Title	Associate Professor
Affiliation	Ulsan National Institute of Science and Technology

▶ **Contact Information**

Address	UNIST-gil 50, Bldg #110, Room #301-7, Ulsan, 44919
Email	seminlee@unist.ac.kr

Research Interest

Cancer genomics & single-cell genomics

Educational Experience

2003	B.S. in Biological Sciences, Seoul National University, Korea
2004	M.S. in Bioinformatics, KAIST, Korea
2007	Ph.D. in Bioinformatics, University of Cambridge, UK

Professional Experience

2011-2016	Research Fellow, Department of Biomedical Informatics, Harvard Medical School USA
2016-	Associate Professor, Department of Biomedical Engineering, UNIST, Korea

Selected Publications (5 maximum)

1. Ji Hoon Phi, Ae Kyung Park, Semin Lee, et al. Genomic analysis reveals secondary glioblastoma after radiotherapy in a subset of recurrent medulloblastomas, Acta Neuropathologica, 2018, Jun;135(6):939-953.
2. Cancer Genome Atlas Research Network. Integrated genomic and molecular characterization of cervical cancer. Nature. 2017 Jan 23.
3. Xi R, Lee S, Xia Y, Kim TM, Park PJ. Copy number analysis of whole-genome data using BIC-seq2 and its application to detection of cancer susceptibility variants. Nucleic Acids Res. 2016 Jul 27;44(13):6274-86.
4. Lodato MA, Woodworth MB, Lee S, Evrony GD, Mehta BK, Karger A, Lee S, Chittenden TW, Cai X, Luquette LJ, Lee E, Park PJ, Walsh CA. Mosaic Mutations Trace Developmental and Transcriptional Histories of Single Human Neurons. Science. 2015 Oct 2;350(6256):94-8.
5. Cancer Genome Atlas Research Network. Comprehensive molecular characterization of human colon and rectal cancer. Nature. 2012 Jul 18;487(7407):330-7.

KSBI | 한국생명정보학회

KSBi-BIML 2024

Single-cell RNA-sequencing analysis of cancer

Semin Lee and Hyoung-oh Jeong

Department of Biomedical Engineering

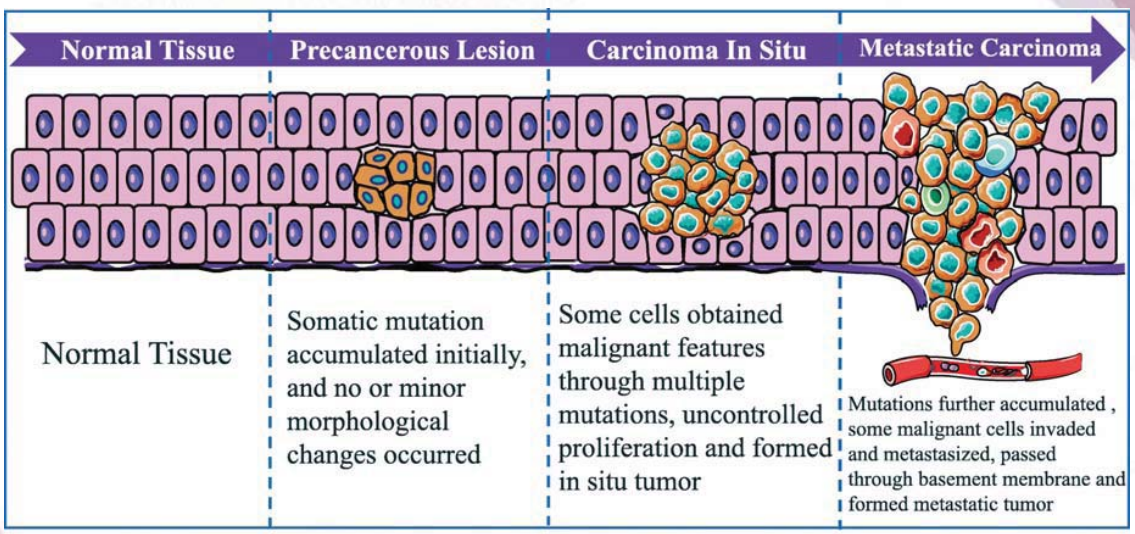
UNIST

1

Introduction

2

What is cancer?

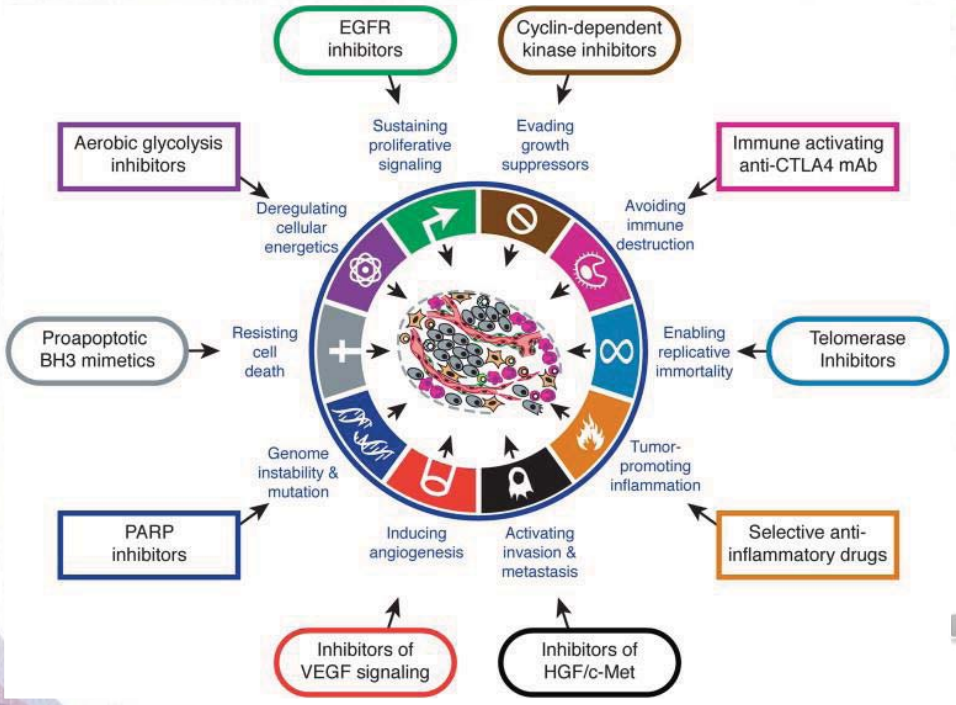


J Exp Clin Cancer Res. 2021

- Cancer is a disease in which some of the body's cells grow uncontrollably and spread to other parts of the body.
- Tumor development is a complex and multi-stage process whereby normal cells develop into malignant tumors, through a series of multiple gene mutations and accumulation in somatic cells.

3

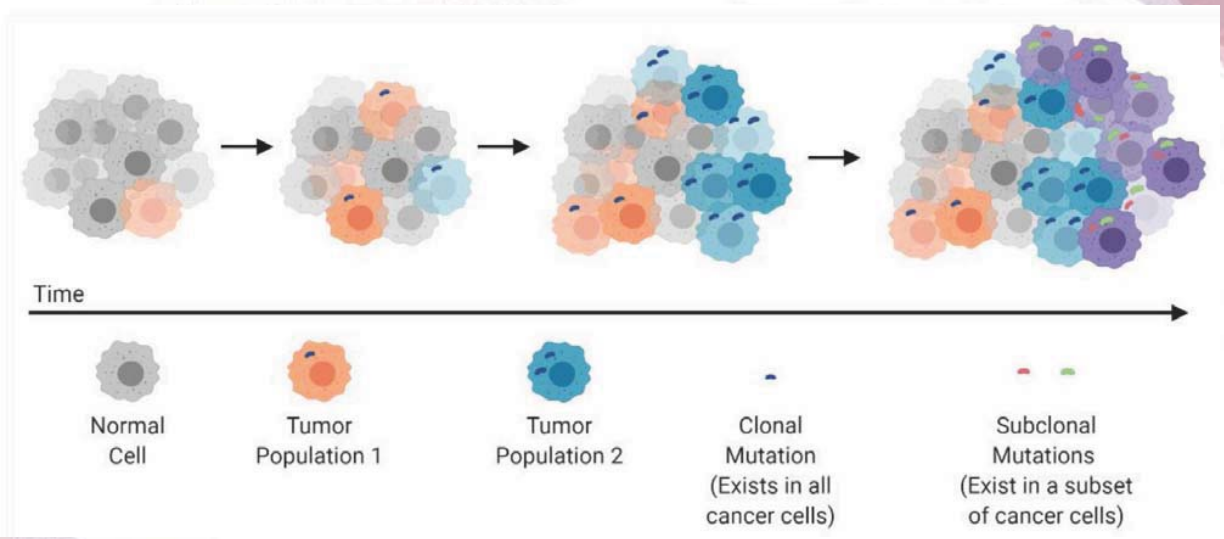
Hallmarks of cancer



Cell. 2011

4

Intratumoral heterogeneity (ITH)

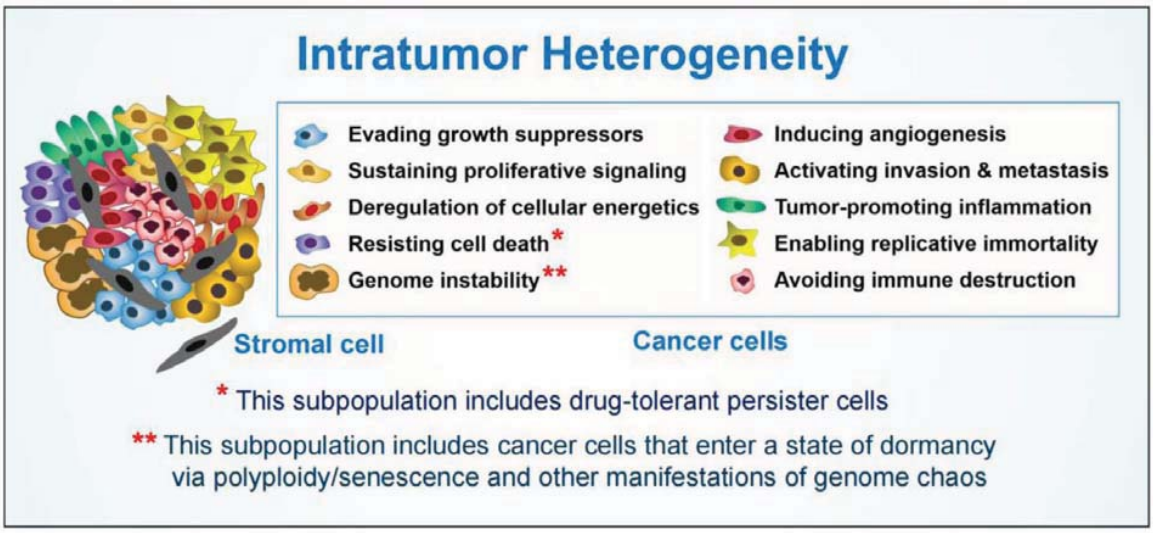


Cancers (Basel). 2021

- Intratumor heterogeneity consists of a single tumor mass which contains several distinct subpopulations of cells, each behaving differently with varied responses to therapeutic intervention

5

ITH and cancer hallmarks

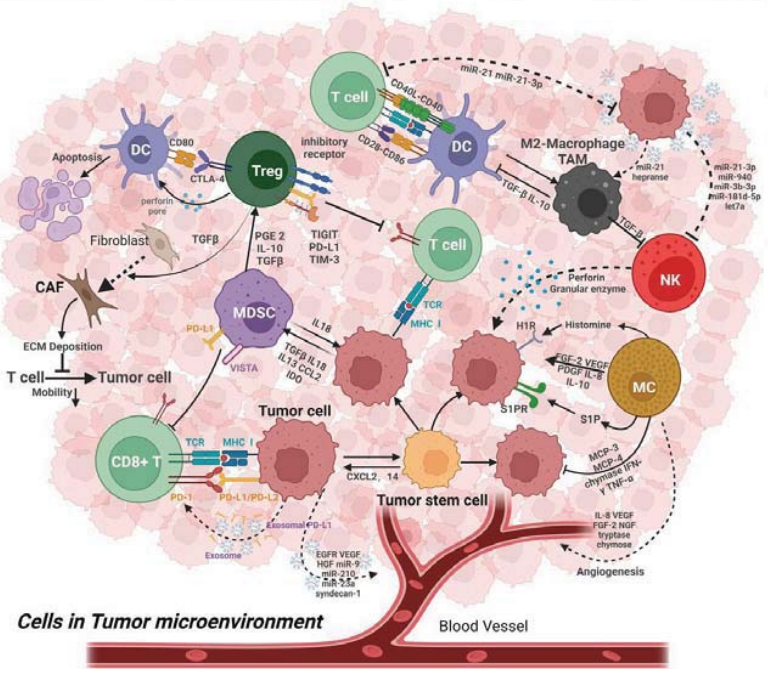


Int J Mol Sci. 2023

- Different subpopulations of cancer cells within a solid tumor/tumor-derived cell line can exhibit therapy resistance via different molecular and cellular processes.

6

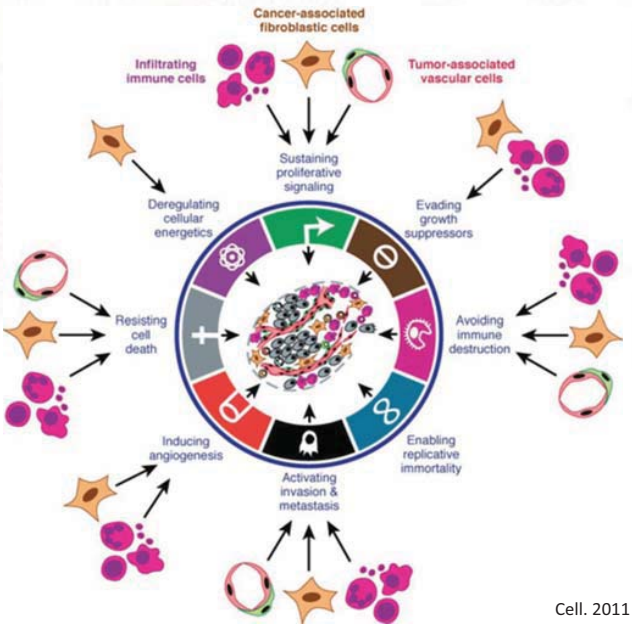
Tumor microenvironment (TME)



Cancer Med. 2023

- The tumor microenvironment is a complex ecosystem surrounding a tumor, composed of a variety of non-cancerous cells including blood vessels, immune cells, fibroblasts, signaling molecules and the extracellular matrix.

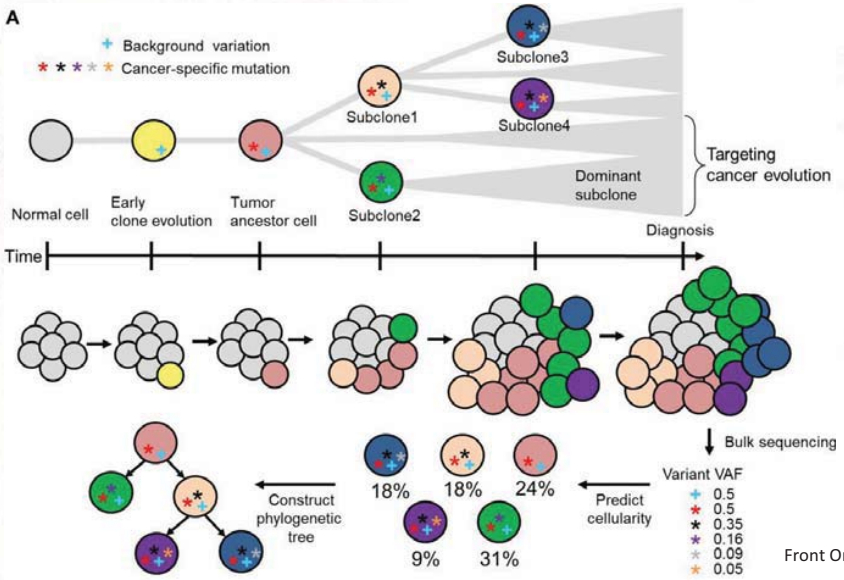
TME and cancer hallmarks



Cell. 2011

- Dynamic and mutualistic interactions between tumor and TME are the distinctive hallmarks of cancer.
- Biochemical and physical cues from the TME serve an essential role in regulating tumor onset and progression.

How to interrogate ITH? : Bulk tissue DNA sequencing

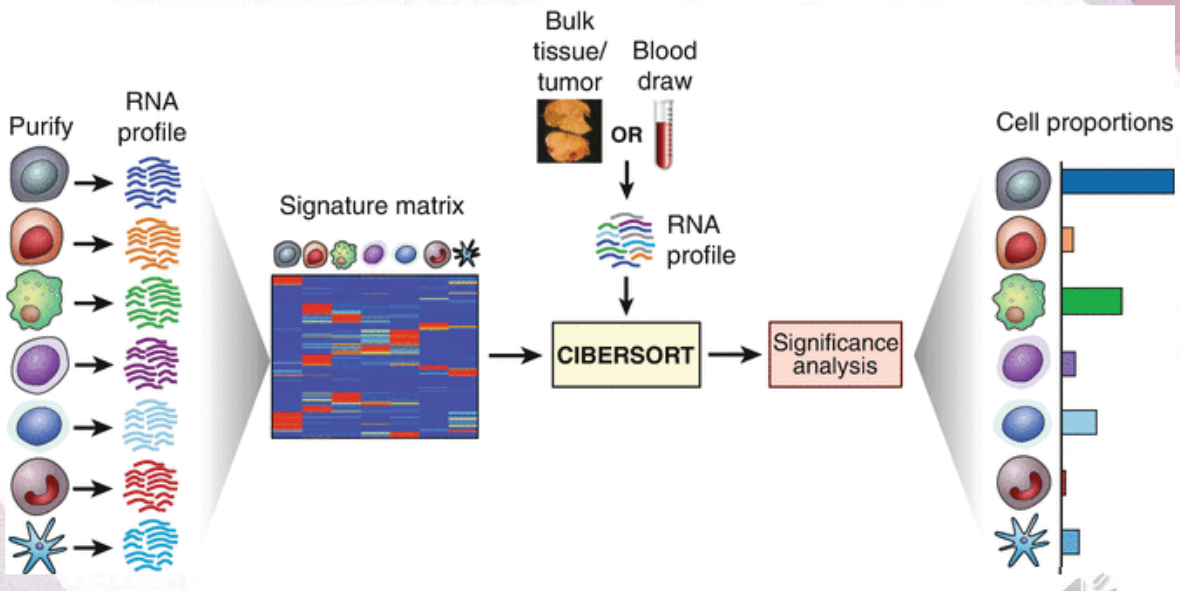


Front Oncol. 2020

- The data of variant allele fraction (VAF) generated from deep sequencing of bulk tumor tissues could be used to predict the cellularity and construct the phylogenetic tree.

9

How to interrogate TME? : Bulk tissue RNA sequencing



Nat Methods. 2015

- Computational deconvolution techniques could help infer the cellular composition of tumors, but such analyses are limited to a few known cell types.

10

Limitations of bulk tissue sequencing

- Bulk sequencing methods are limited to reporting an average signal from a complex population of cells.
- So, it is difficult to resolve cell-to-cell variations in ITH and identify the complex nature of TME.
- Deep bulk tissue DNA/RNA sequencing could help infer the clonal architecture and cellular composition of tumor and its microenvironment, but such analyses are limited to a few dominant subclones and known cell types.

11

Advantages of single-cell RNA sequencing

- Single-cell RNA sequencing (scRNA-seq)
  - not only identifies ITH but also reconstruct a high-resolution map of the TME.
  - identifies cell-specific gene expression profiles and genetic variants
  - can reconstruct tumor clonality and evolution.
- scRNA-seq does not rely on known cell type-specific gene signatures or surface markers.

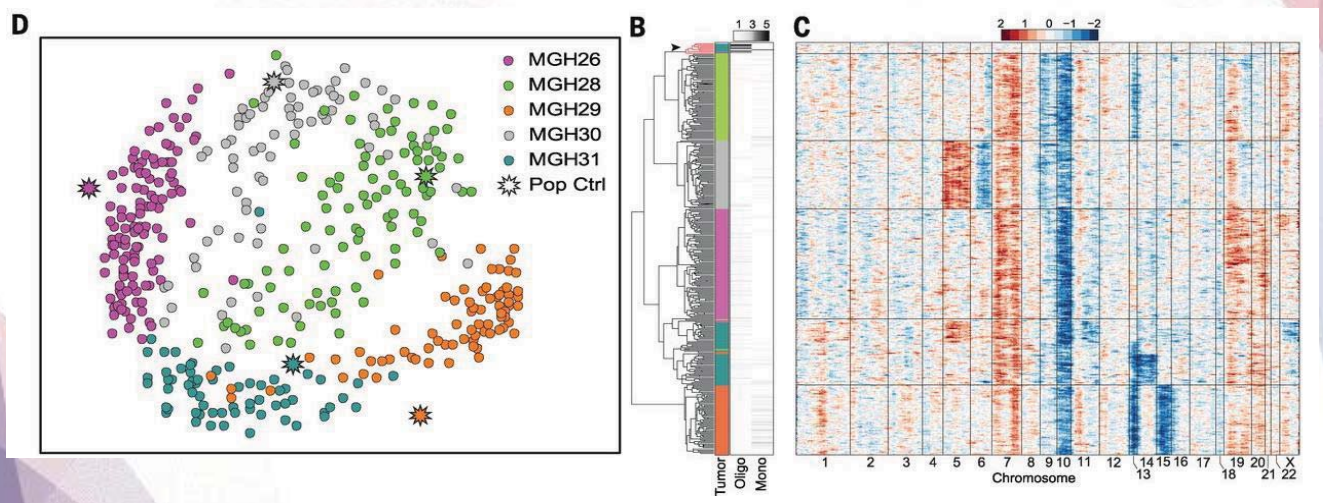
12

Applications of scRNA-seq in cancer studies

- Tumor heterogeneity
- Clonal evolution of cancer
- Tumor microenvironment
- Circulating tumor cells

13

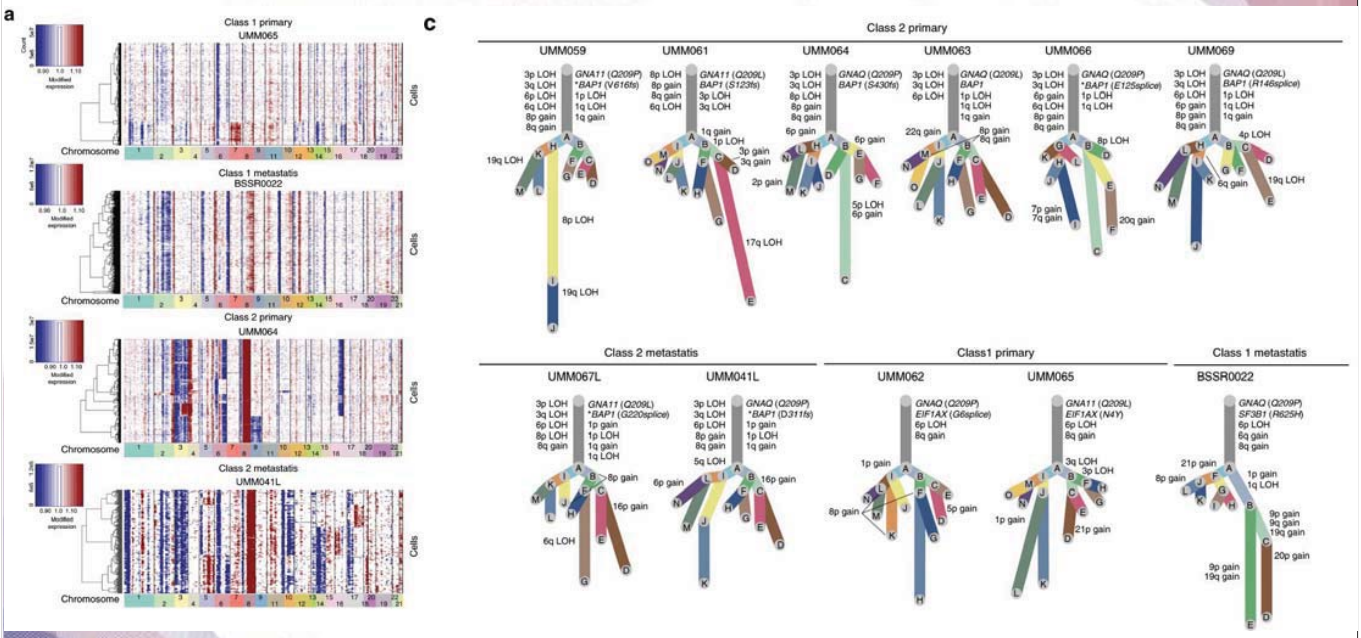
Applications of scRNA-seq : Tumor heterogeneity



Science. 2014

14

Applications of scRNA-seq : Clonal evolution of cancer



Nat Commun. 2020

15

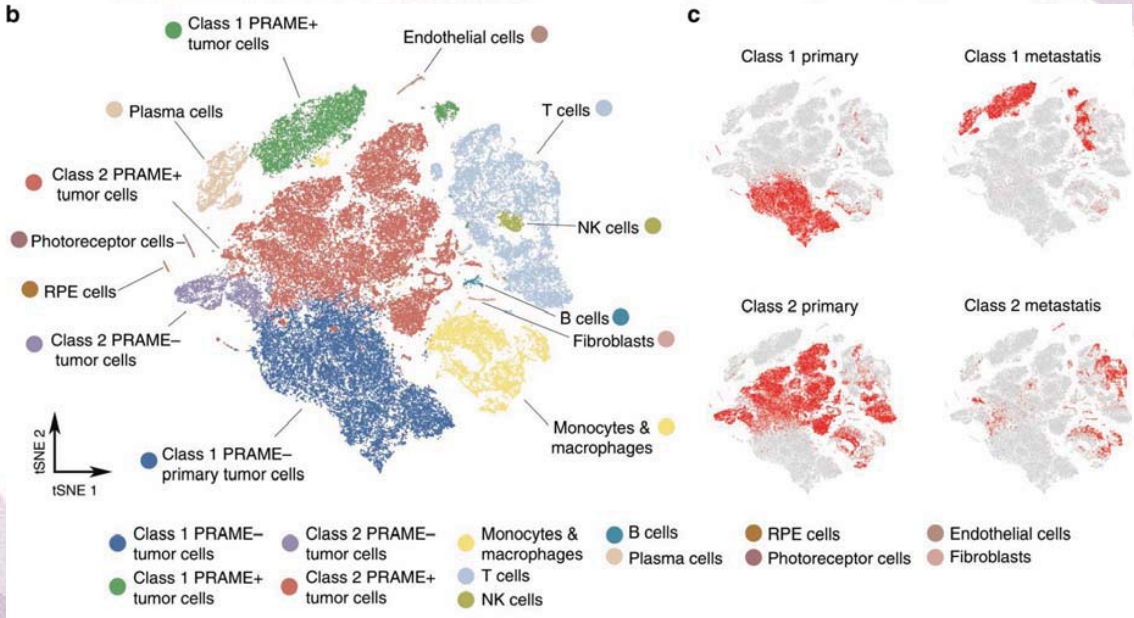
Applications of scRNA-seq : Circulating tumor cells

Cancer type	CTC enrichment	CTC criteria (micromanipulation)	Single-cell profiling	Number of CTCs (number of patients)[a]
Multiple myeloma	FACS with serial dilution	$CD45^-$, $CD138^+$	SMART-seq2	21 (2)
Colon	CellSearch®	$CD45^-$, $EpCAM^+$	Multiplex PCR	11 (8)
Ovary	Biocoll separation, Dynabeads® CD45 depletion	$DAPI^+$, $CK/EpCAM^+$, $CD45^-$	Multiplex PCR	15 (3)
Breast	MagSweeper®	$EpCAM^+$	Microfluidic RT-PCR[b]	105 (50)
	Microfluidic ^{neg}CTC-iChip	$EpCAM/HER2/CDH11^+$, $CD45/CD16/CD14^-$	Optimized Tang's method	15 (10)
	Microfluidic CTC-iChip	$EpCAM/HER2/EGFR^+$, $CD45^-$	SMART-seq v4[c]	15 (10)
	Microfluidic ClearCell® FX	$CD45/CD31^-$, $Calcein^+$ [d]	Polaris™ IFC	68 (4)
Melanoma	MagSweeper®	$CD45^-$, $Calcein^+$	SMART-seq	6 (1)
Prostate	MagSweeper®	$CD45^-$, $EpCAM^+$, $DAPI^-$	SMART-seq, Advantage 2 PCR (Clontech)	20 (4)
	ScreenCell®	$CD45^-$	Microfluidic RT-PCR[e]	38 (9)
	Microfluidic CTC-iChip	$CD45^-$, $EpCAM/CDH11^+$	Modified Tang's method	77 (13)
Lung	Integrated nanoplatform	$EpCAM^+$	Multiplex PCR	8 (1), 18 (1), 74 (1)
	Microfluidic ClearCell® FX	$CD45^-$ [f]	Multiplex PCR	61 (20)
Prostate, breast	CellSearch®, Parsortix™	$EpCAM/pan\text{-}keratins^+$	Multiplex PCR	13 (1), 8 (1)
Pancreas, breast, prostate	Microfluidic CTC-iChip	$CD45^-$	Modified Tang's method	7 (–), 29 (–), 77 (–)

NPJ Precis Oncol. 2019

16

Applications of scRNA-seq : Tumor microenvironment



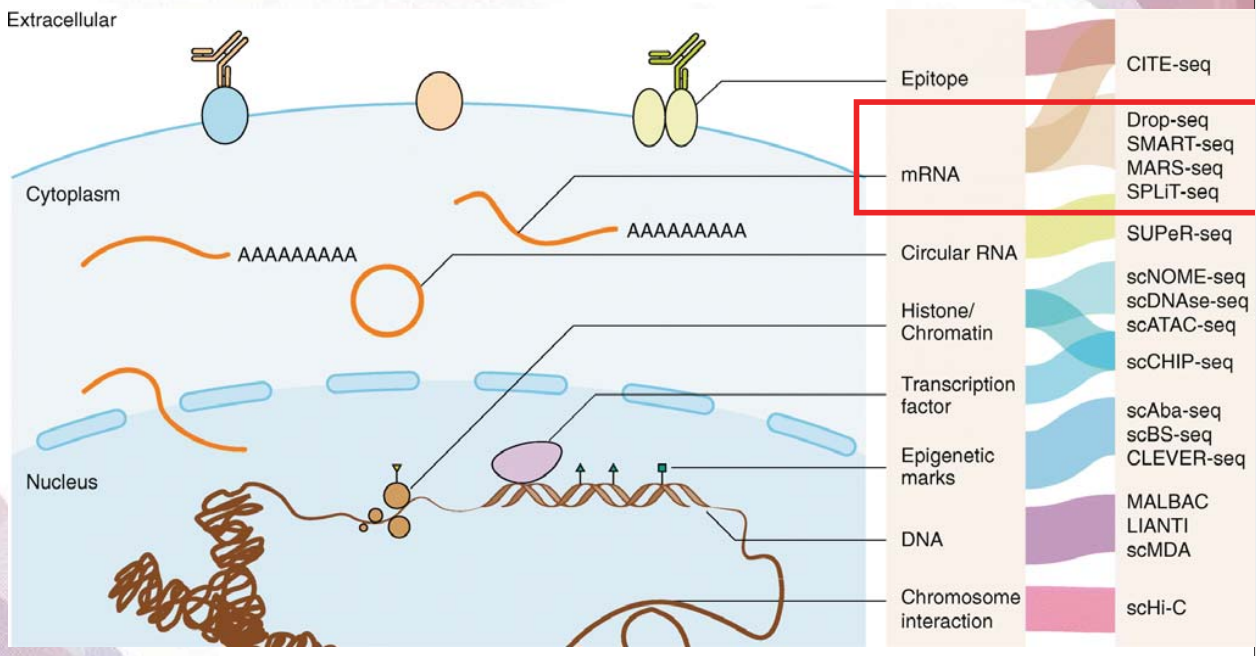
Nat Commun. 2020

17

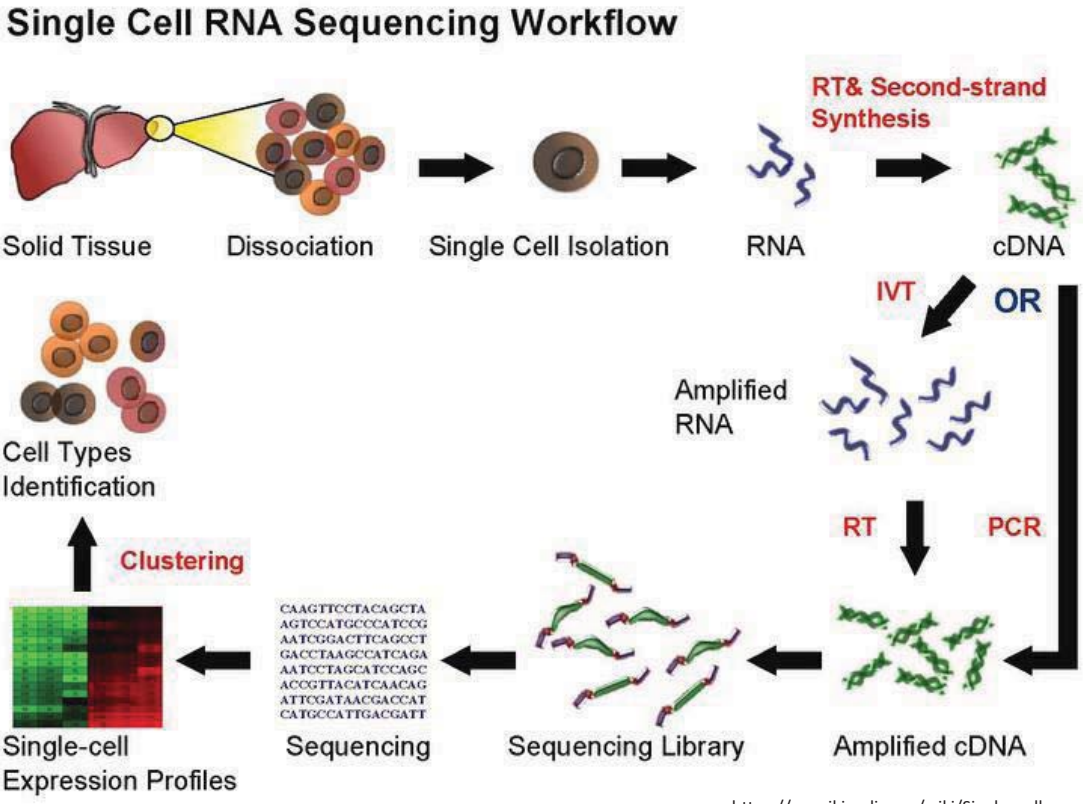
Single-cell sequencing

18

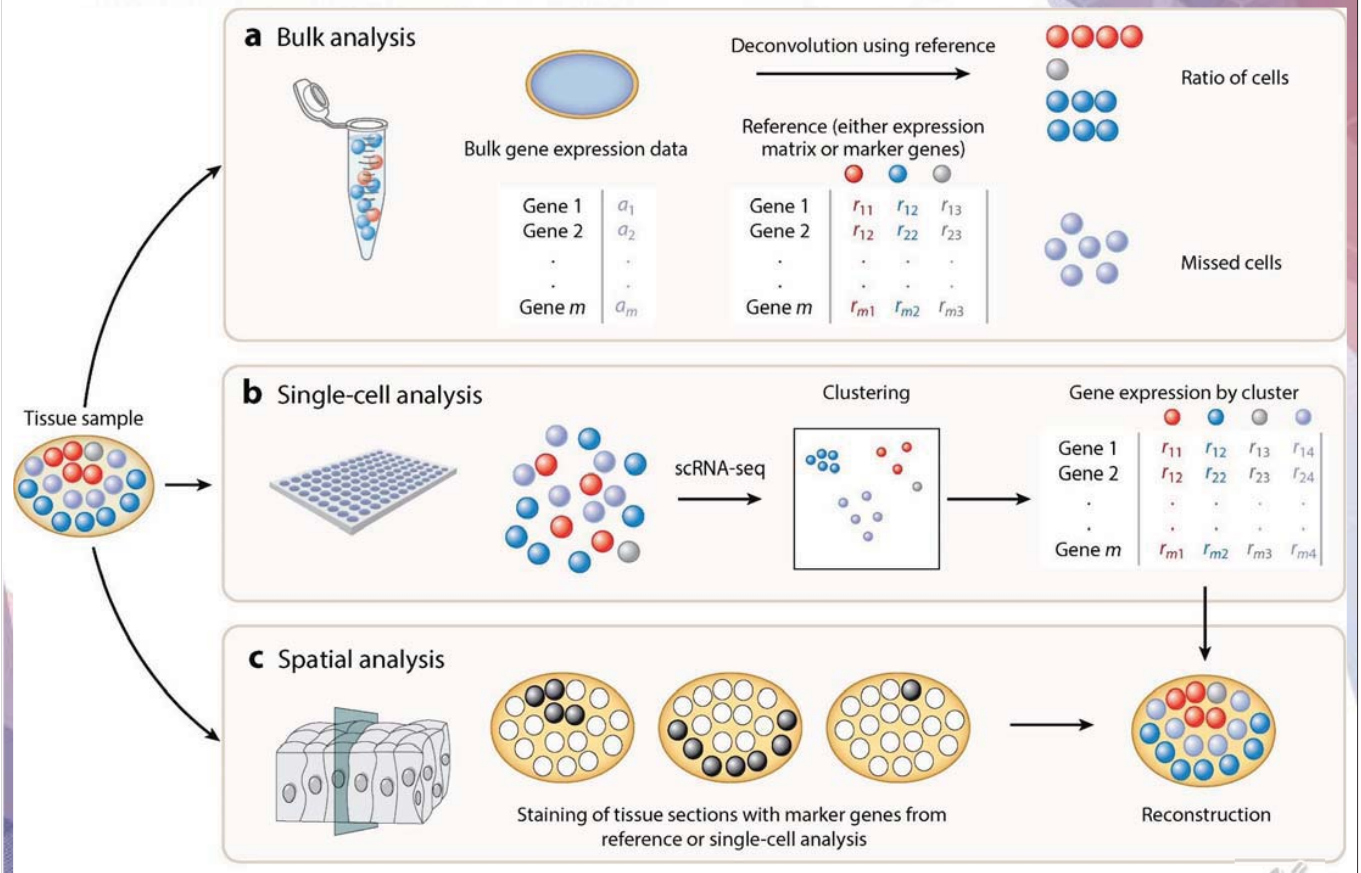
State of the art of single-cell sequencing technologies



Genome Biol. 2015



https://en.wikipedia.org/wiki/Single_cell_sequencing

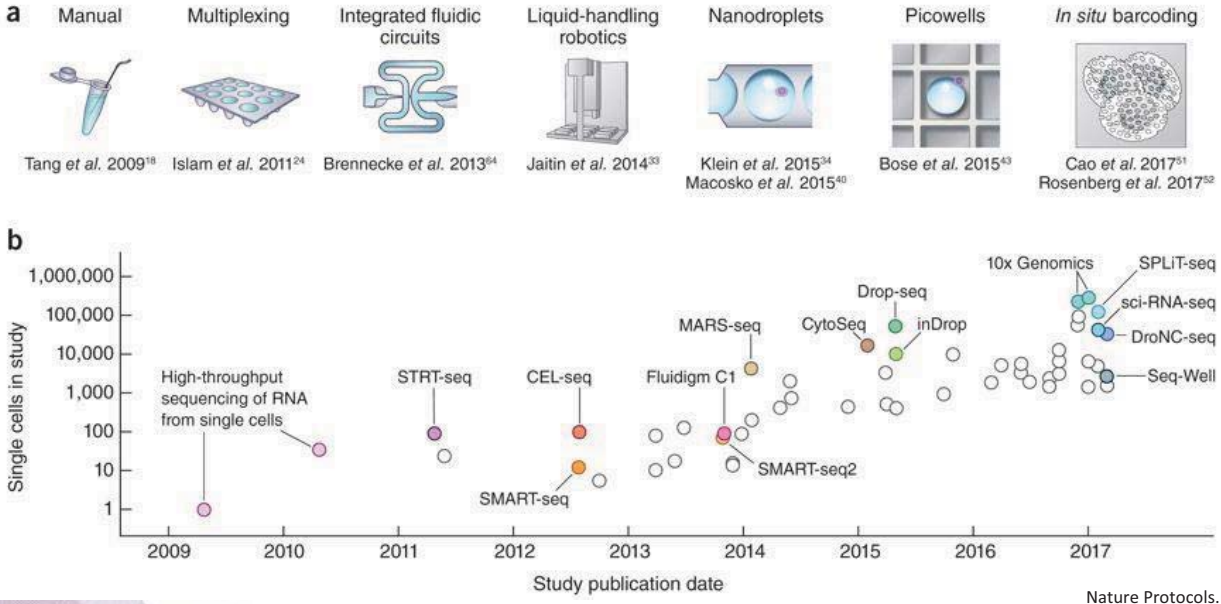


Chen X, et al. 2018.
Annu. Rev. Biomed. Data Sci. 1:29–51

Annual review of biomedical data science. 2018

21

Scaling of scRNA-seq experiments



Nature Protocols. 2018

22

REPORT

Defining the earliest step of cardiovascular lineage segregation by single-cell RNA-seq

Fabienne Lescroart[1,*], Xiaonan Wang[2,3,*], Xionghui Lin[1,*], Benjamin Swedlund[1], Souhir Gargouri[1], Adriana Sànchez-Dànes[1], ...
+ See all authors and affiliations

Science 09 Mar 2018:
Vol. 359, Issue 6380, pp. 1177-1181
DOI: 10.1126/science.aao4174

REPORT

Single-cell multiomics sequencing and analyses of human colorectal cancer

Shuhui Bian[1,2,3,*], Yu Hou[1,2,*], Xin Zhou[4,*], Xianlong Li[1,2,*], Jun Yong[1,5,*], Yicheng Wang[1,2,*], Wendong Wang[4], Jia Yan[1,2], Bo...
+ See all authors and affiliations

Science 30 Nov 2018:
Vol. 362, Issue 6418, pp. 1060-1063
DOI: 10.1126/science.aao3791

RESEARCH ARTICLE

Decoupling genetics, lineages, and microenvironment in IDH-mutant gliomas by single-cell RNA-seq

Andrew S. Venteicher[1,2,3,*], Itay Tirosh[2,*,†], Christine Hebert[1,2], Keren Yizhak[1,2], Cyril Neftel[1,2,4], Mariella G. Filbin[1,2,5], Volk...
+ See all authors and affiliations

Science 31 Mar 2017:
Vol. 355, Issue 6332, eaai8478
DOI: 10.1126/science.aai8478

nature genetics

Article | Published: 11 March 2019

Interrogation of human hematopoiesis at single-cell and single-variant resolution

Jacob C. Ulirsch, Caleb A. Lareau, Erik L. Bao, Leif S. Ludwig, Michael H. Guo, Christian Benner, Ansuman T. Satpathy, Vinay K. Kartha, Rany M. Salem, Joel N. Hirschhorn, Hilary K. Finucane, Martin J. Aryee, Jason D. Buenrostro & Vijay G. Sankaran

Nature Genetics **51**, 683–693 (2019) | Download Citation

nature
International Journal of Science

Letter | Published: 14 March 2018

A single-cell RNA-seq survey of the developmental landscape of the human prefrontal cortex

Suijuan Zhong, Shu Zhang, Xiaoying Fan, Qian Wu, Liying Yan, Ji Dong, Haofeng Zhang, Long Li, Le Sun, Na Pan, Xiaohui Xu, Fuchou Tang, Jun Zhang, Jie Qiao & Xiaoqun Wang

Nature **555**, 524–528 (22 March 2018) | Download Citation

nature medicine

Letter | Published: 25 June 2018

Single-cell profiling of breast cancer T cells reveals a tissue-resident memory subset associated with improved prognosis

Peter Savas, Balaji Virassamy, [...] Sherene Loi

Cell Stem Cell

Volume 17, Issue 3, 3 September 2015, Pages 360-372

CellPress

Resource

Single-Cell RNA-Seq with Waterfall Reveals Molecular Cascades underlying Adult Neurogenesis

Jaehoon Shin, Daniel A. Berg, Yunhua Zhu, Joseph Y. Shin, Juan Song, Michael A. Bonaguidi, Grigori Enikolopov, David W. Nauen, Kimberly M. Christian, Guo-li Ming, Hongjun Song

nature immunology

Resource | Published: 15 February 2016

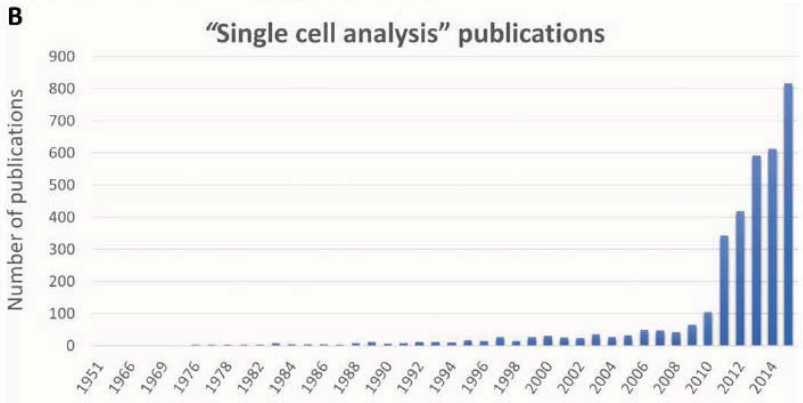
The heterogeneity of human CD127$^+$ innate lymphoid cells revealed by single-cell RNA sequencing

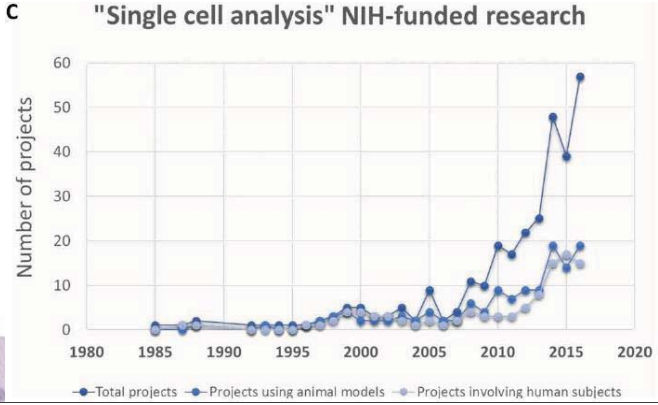
Åsa K Björklund, Marianne Forkel, Simone Picelli, Viktoria Konya, Jakob Theorell, Danielle Friberg, Rickard Sandberg & Jenny Mjösberg

Nature Immunology **17**, 451–460 (2016) | Download Citation

23

Historical trends of single cell analysis

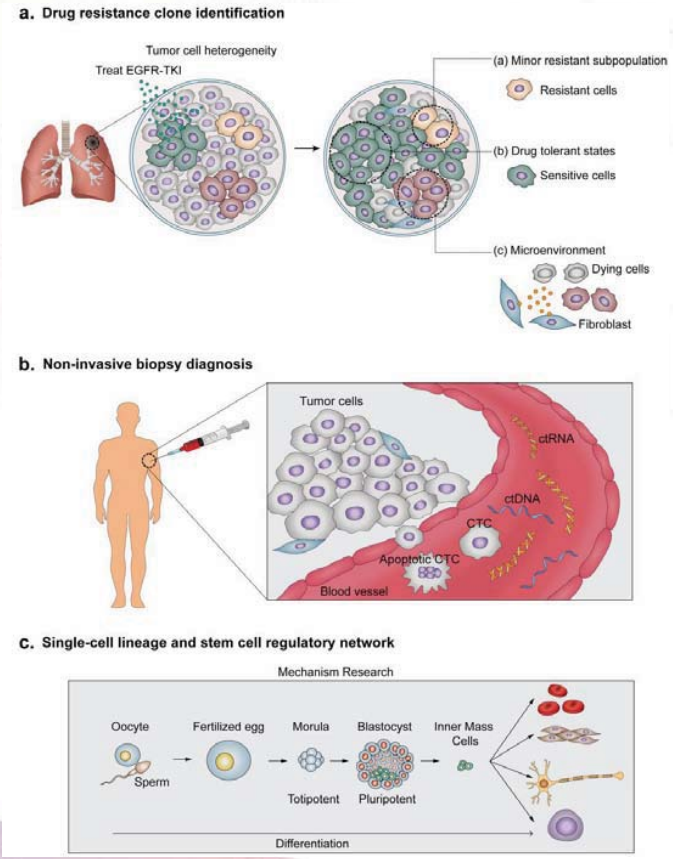




Sci. Adv. 2018

24

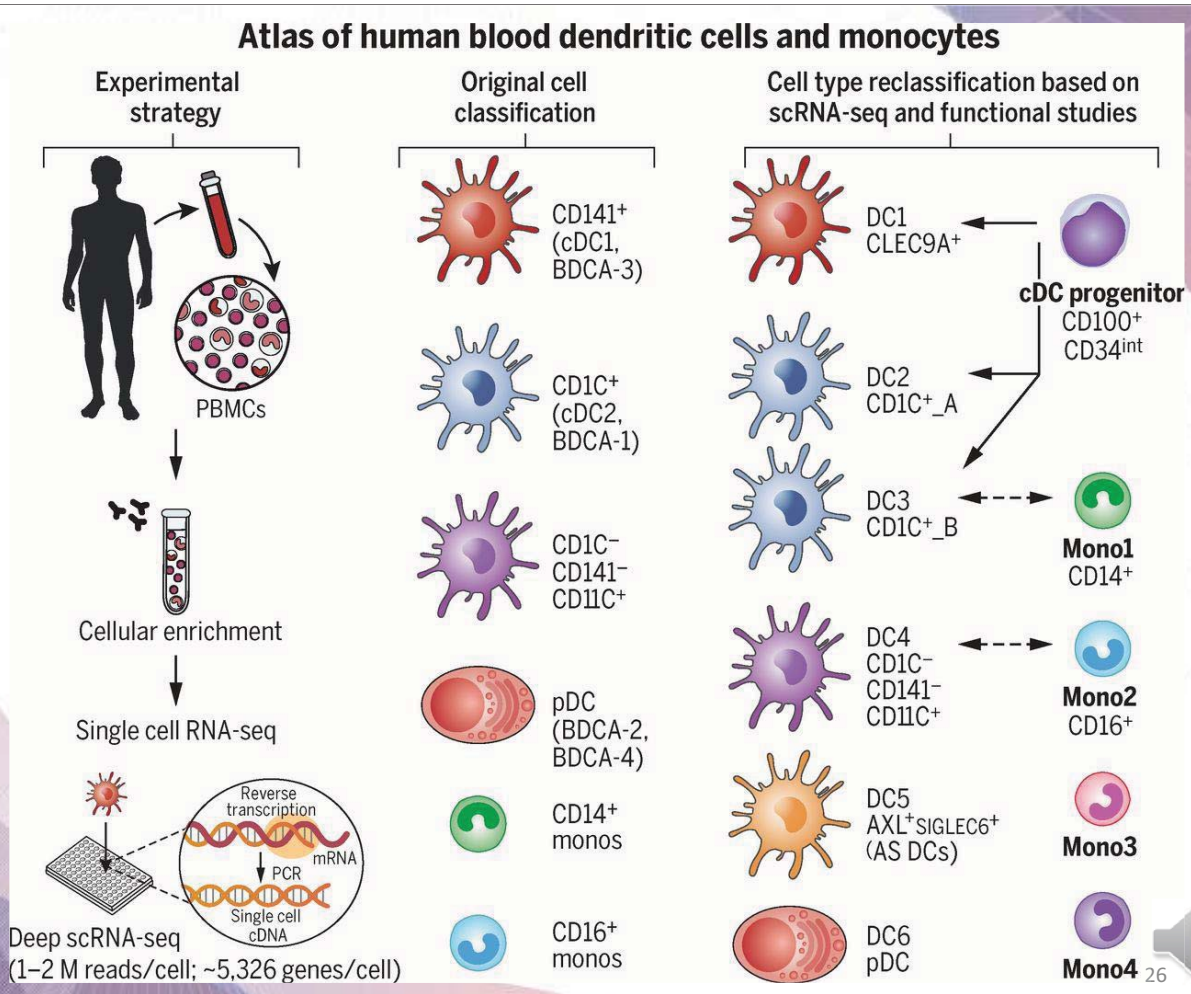
Many facets of scRNA-seq applications



25

Experimental & Molecular Medicine. 2018

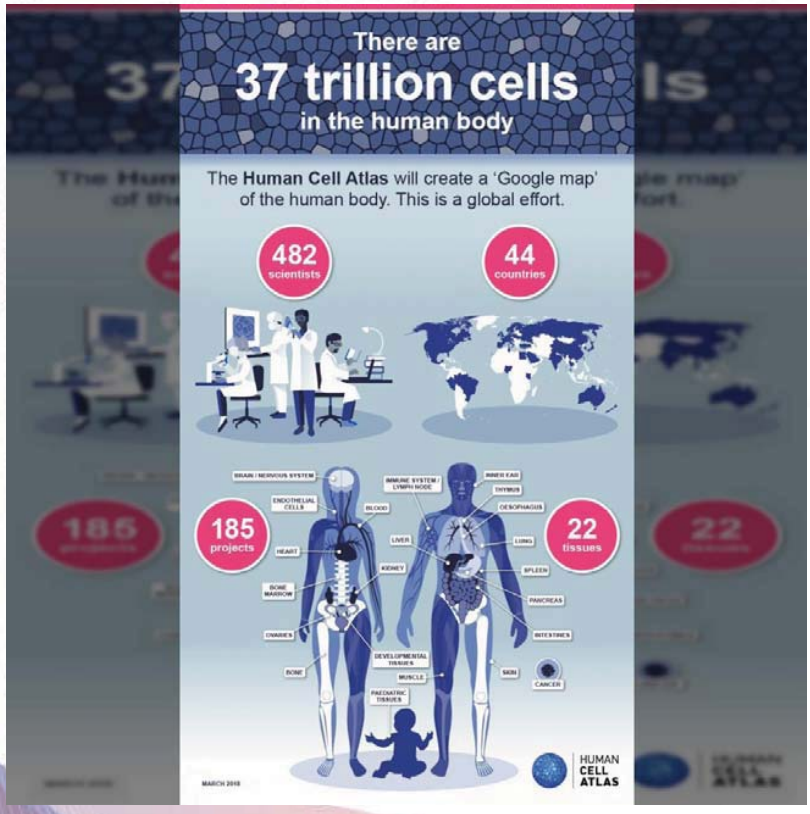
Atlas of human blood dendritic cells and monocytes



26

Science. 2017

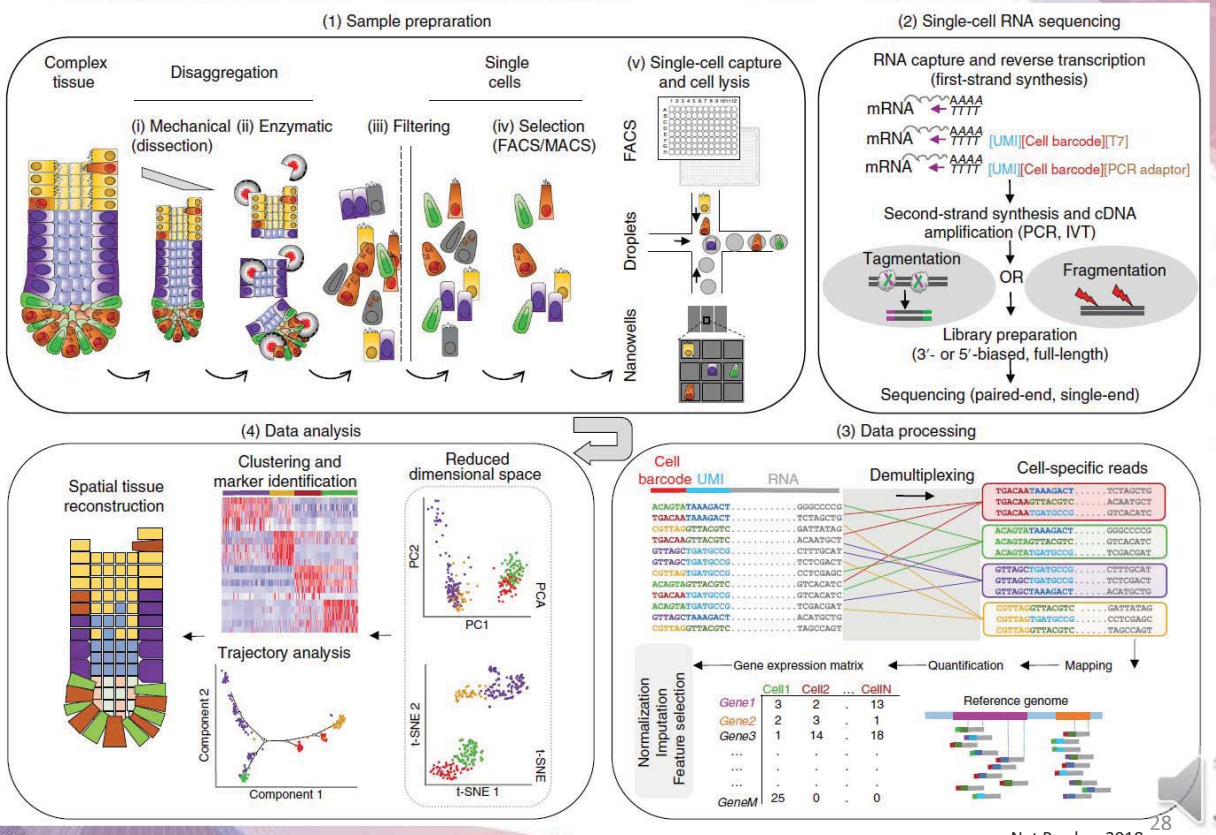
The Human Cell Atlas



27

https://www.humancellatlas.org/

Single-cell RNA sequencing process

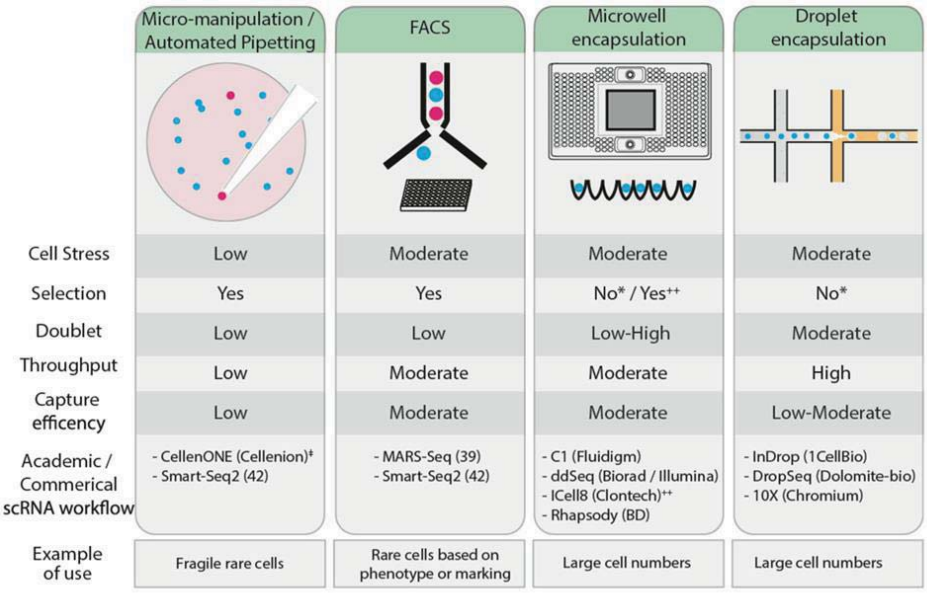


28

Nat Prodoc. 2018

scRNA-seq platforms

29



	Micro-manipulation / Automated Pipetting	FACS	Microwell encapsulation	Droplet encapsulation
Cell Stress	Low	Moderate	Moderate	Moderate
Selection	Yes	Yes	No* / Yes++	No*
Doublet	Low	Low	Low-High	Moderate
Throughput	Low	Moderate	Moderate	High
Capture efficency	Low	Moderate	Moderate	Low-Moderate
Academic / Commerical scRNA workflow	- CellenONE (Cellenion)‡ - Smart-Seq2 (42)	- MARS-Seq (39) - Smart-Seq2 (42)	- C1 (Fluidigm) - ddSeq (Biorad / Illumina) - ICell8 (Clontech)++ - Rhapsody (BD)	- InDrop (1CellBio) - DropSeq (Dolomite-bio) - 10X (Chromium)
Example of use	Fragile rare cells	Rare cells based on phenotype or marking	Large cell numbers	Large cell numbers

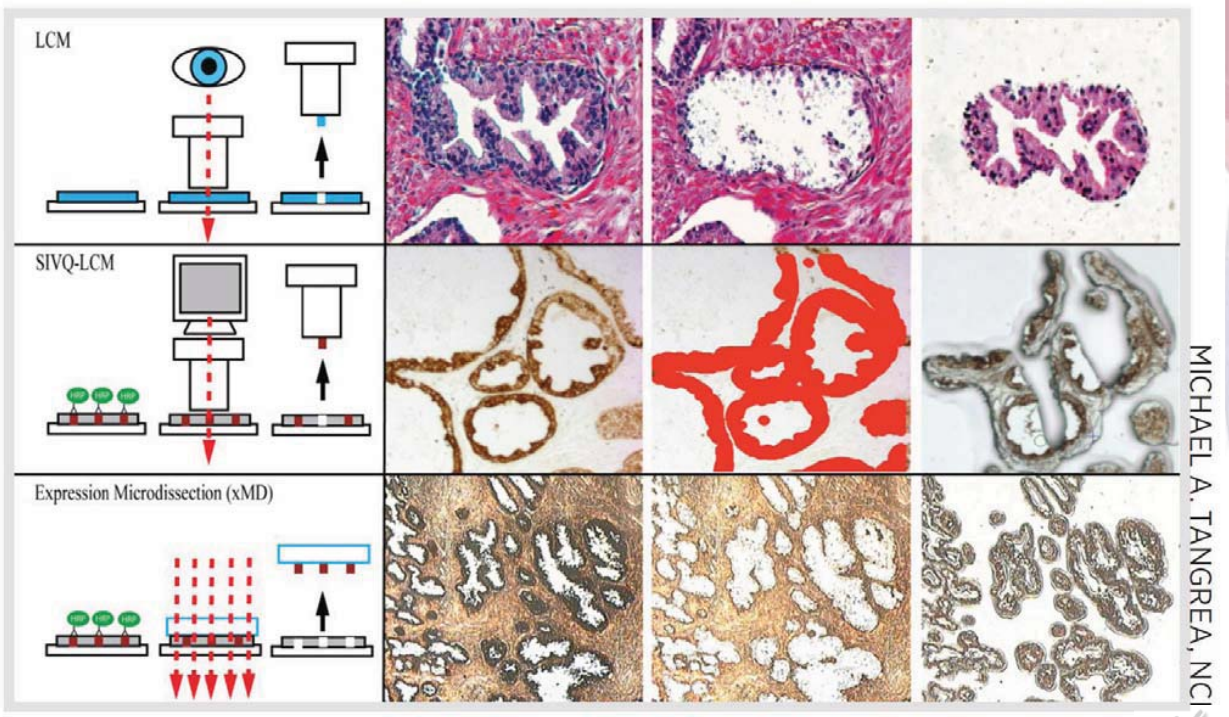
	FACS		Microwell encapsulation				Droplet encapsulation		
	Smart-Seq2	MARS-Seq	C1	ddSeq	ICell8	Rhapsody	InDrop	DropSeq	10X
Singlet Capture efficency	82%	92%	39%	2.6%	37%++	Not reported	7%	Not reported	50%
Doublet rate	Not reported	2.27%	3-30%	5.8%	1.3-4%	0.6%	4%	0.36-11.3	1.6-3%
Reference	42	39	37 FWP	PB	PB	PB	36	37	26

Front. Immunol. 2018

‡Automated pipetting system
*Preselection or enrichment can be performed prior
++Only reagents added to wells containing singlets, determined by system
FWP: Fluidigm white paper
PB: Product brochure / manual

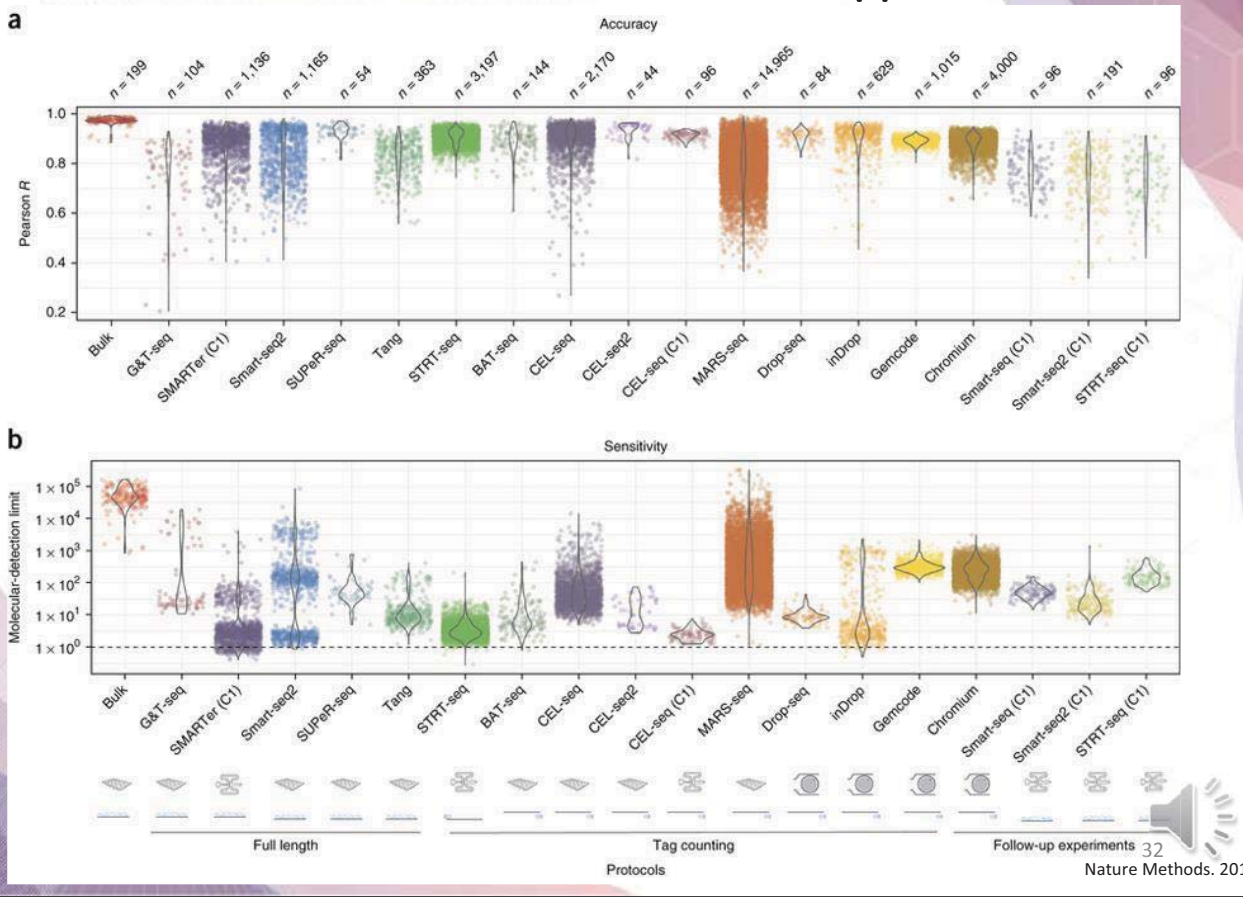
30

Laser capture microdissection (LCM)



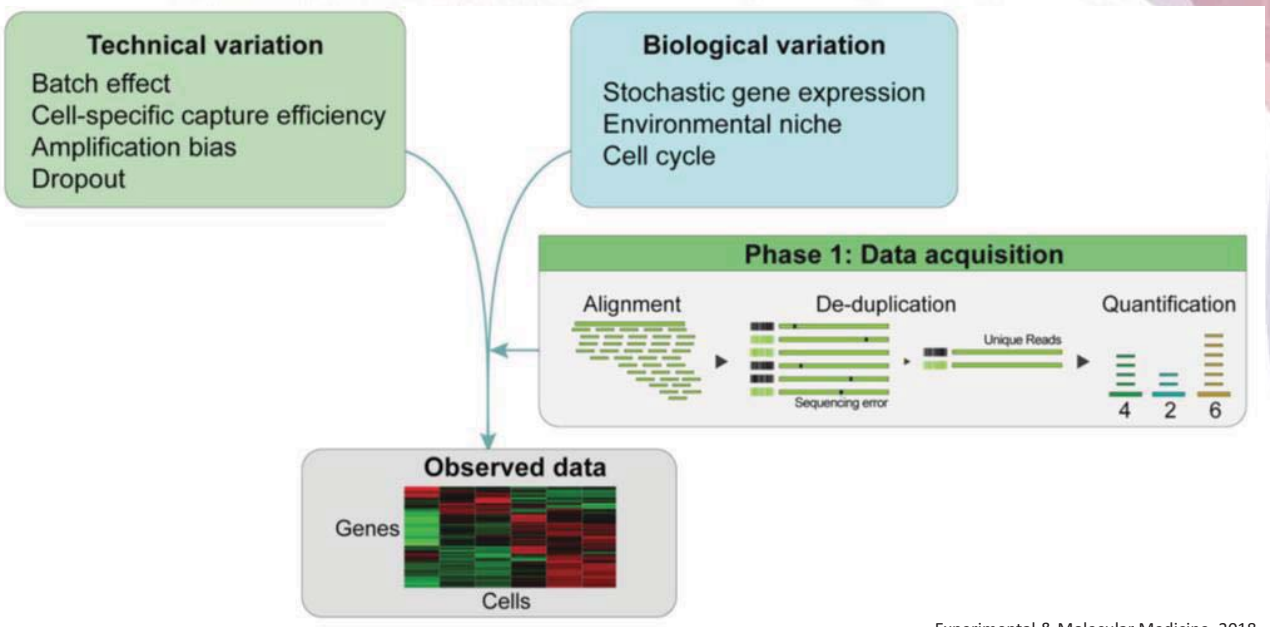
https://irp.nih.gov/catalyst/v19i6/new-methods

Performance metrics for scRNA-seq protocols



Nature Methods. 2017

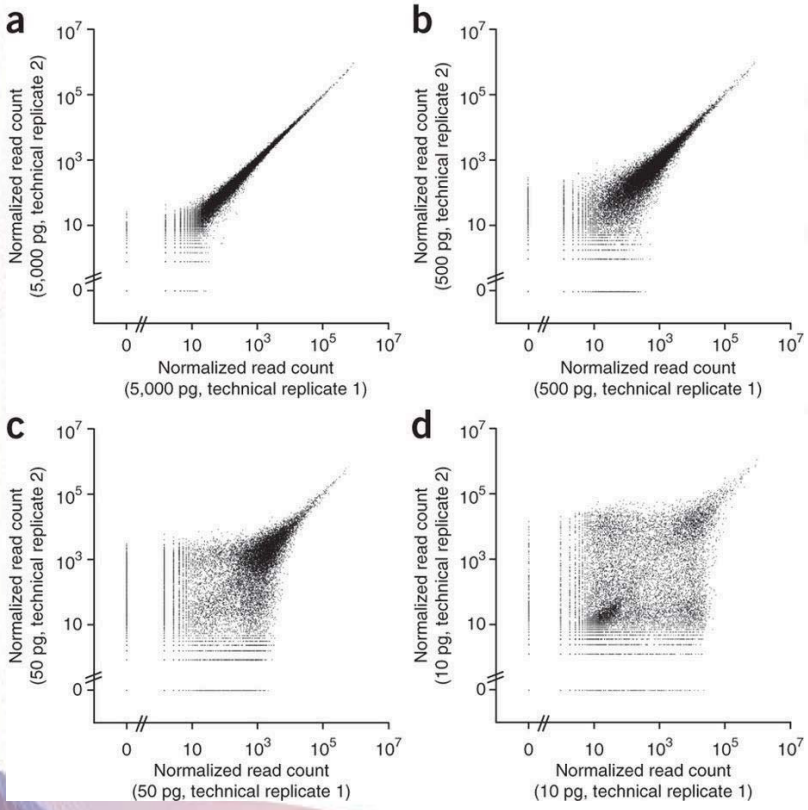
Technical noise in scRNA-seq



Experimental & Molecular Medicine. 2018

33

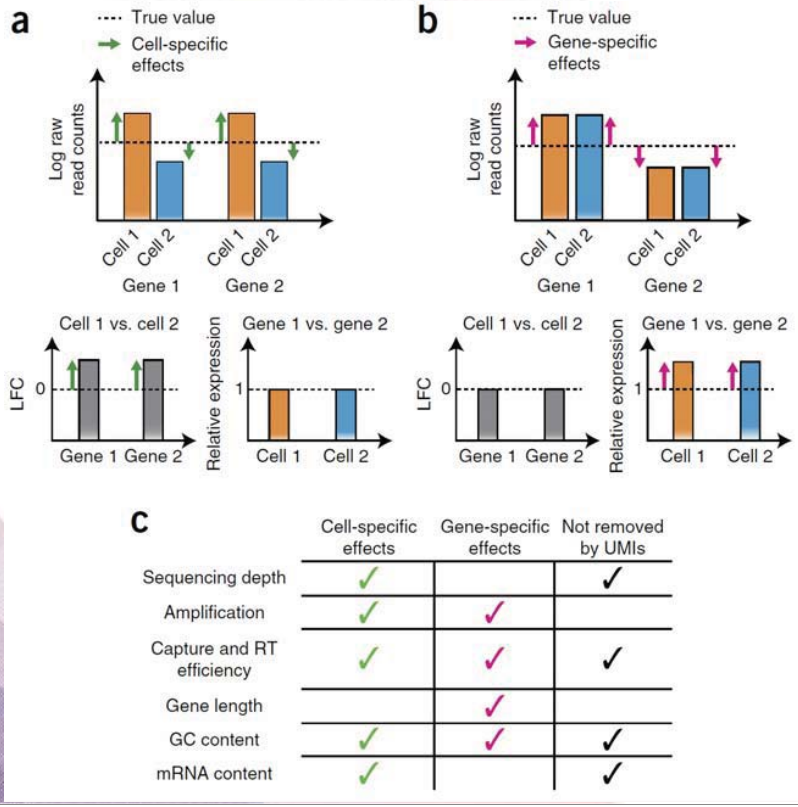
Technical noise in scRNA-seq



34

Nature Methods. 2013

Cell and gene-specific effects in scRNA-seq experiments



c

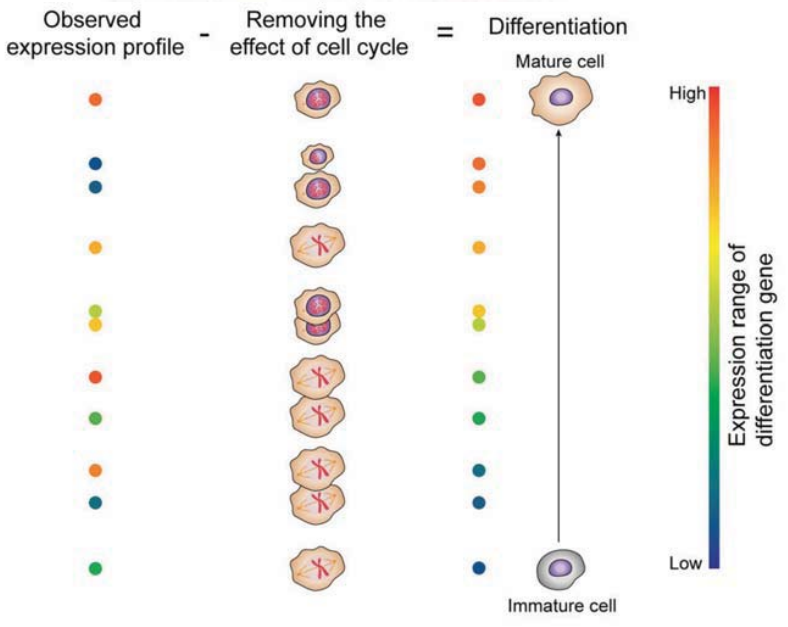
	Cell-specific effects	Gene-specific effects	Not removed by UMIs
Sequencing depth	✓		✓
Amplification	✓	✓	
Capture and RT efficiency	✓	✓	✓
Gene length		✓	
GC content	✓	✓	✓
mRNA content	✓		✓

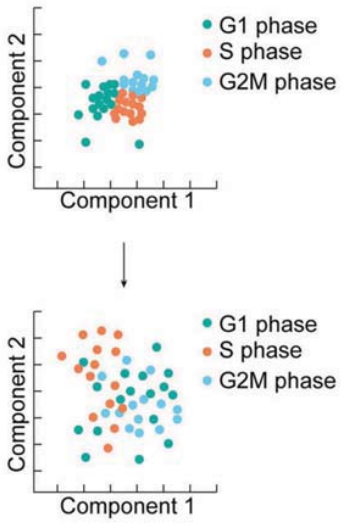
- There are several experimental sources of systematic biases that can affect measurements of gene expression, including gene- and cell-specific features

Nature Methods. 2017

35

Technical noise in scRNA-seq

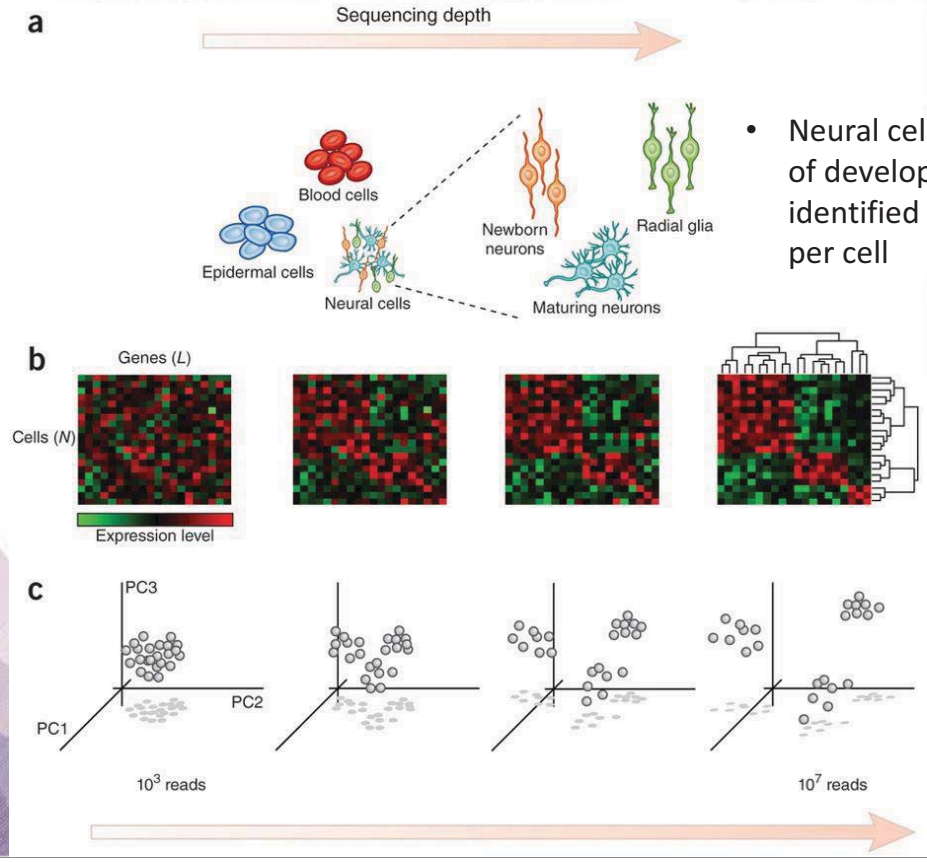




Experimental & Molecular Medicine. 2018

36

The effect of sequencing depth

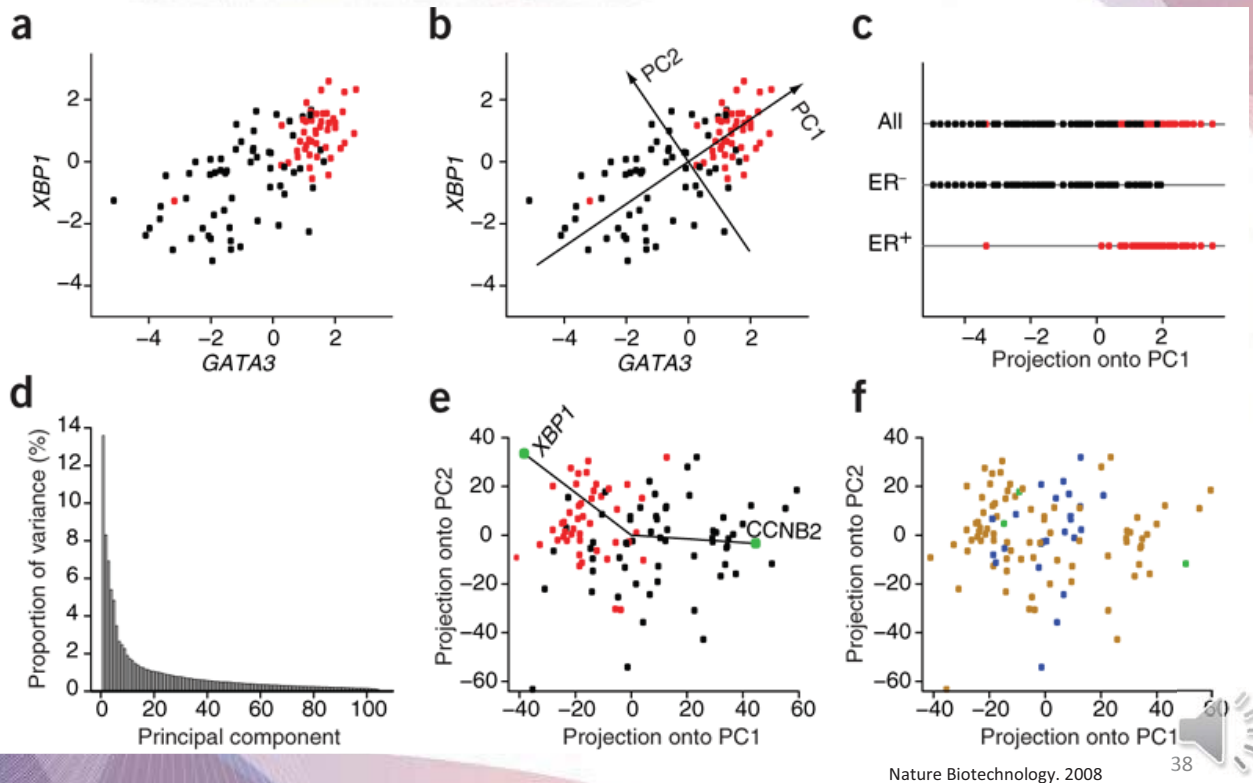


- Neural cells at various stages of development can be identified with ~50,000 reads per cell

Nature Biotechnology. 2014

37

Principal Component Analysis (PCA)



Nature Biotechnology. 2008

38

T-distributed stochastic neighborhood embedding (t-SNE)

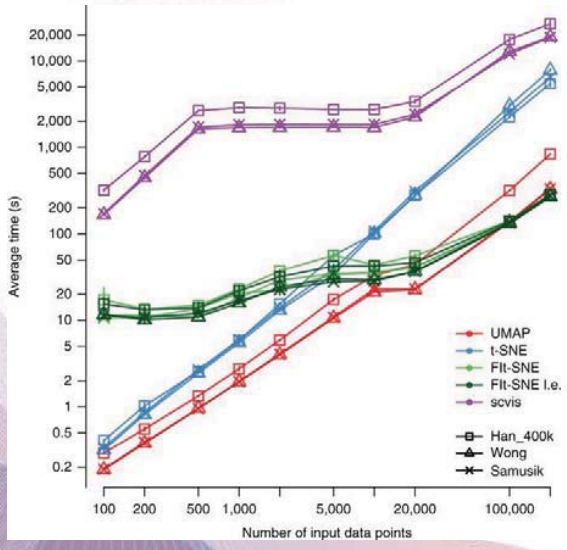
- PCA has historically been the most commonly used method for dimensionality reduction.
- The importance of nonlinear dimensionality reduction techniques has recently been recognized.
  - able to avoid overcrowding of the representation
  - Isomap, Diffusion Map and t-SNE
- t-SNE is currently the most commonly used technique in single-cell analysis
  - t-SNE suffers from limitations such as loss of large-scale information
  - slow computation time
  - inability to meaningfully represent very large datasets

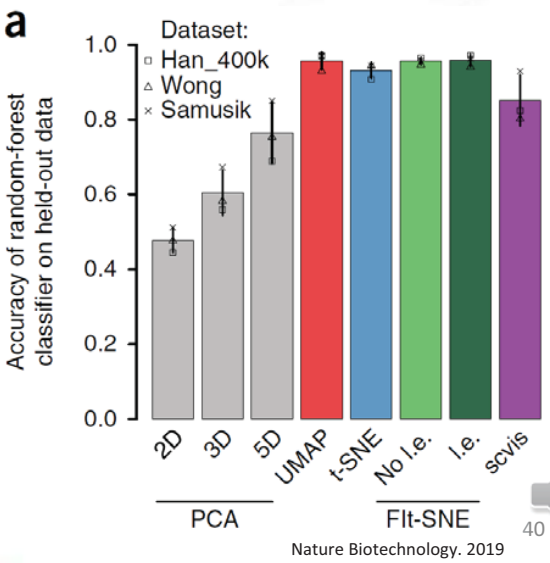
Nature Biotechnology. 2019

39

Uniform manifold approximation and projection (UMAP)

- UMPA preserve as much of the local and more of the global data structure than t-SNE, with a shorter run time.

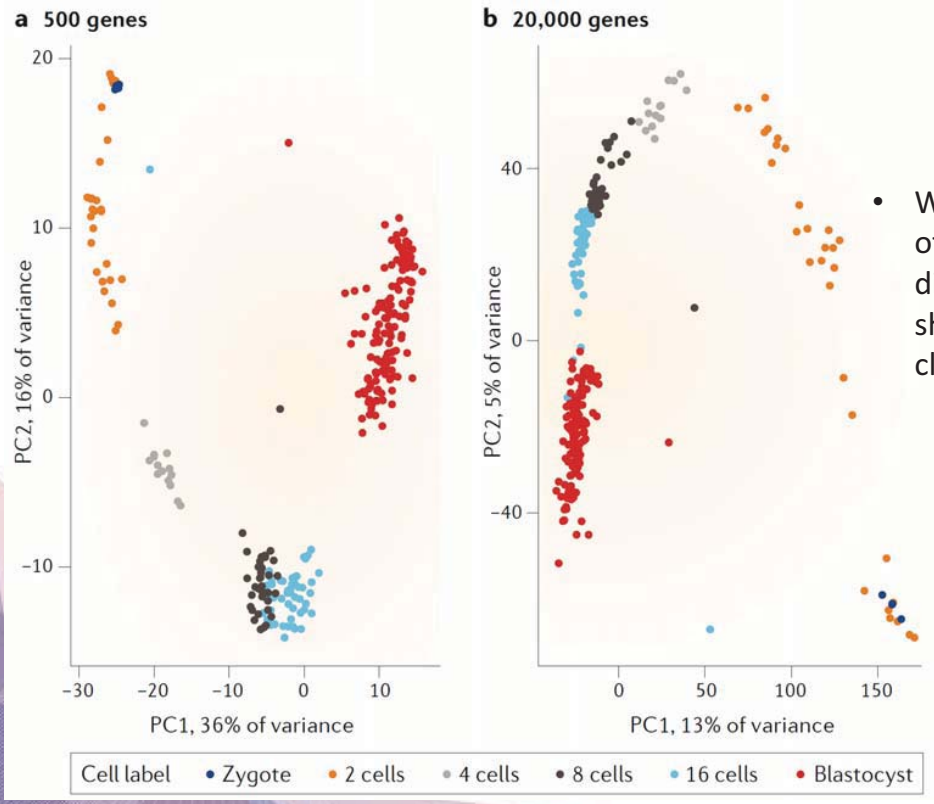




40

Nature Biotechnology. 2019

The curse of dimensionality



- When using a large number of features, clusters are less distinct, as indicated by the shorter distances between clusters

Nature Reviews. 2019

41

Clustering analysis

Table 1 | **Clustering methods for scRNA-seq**

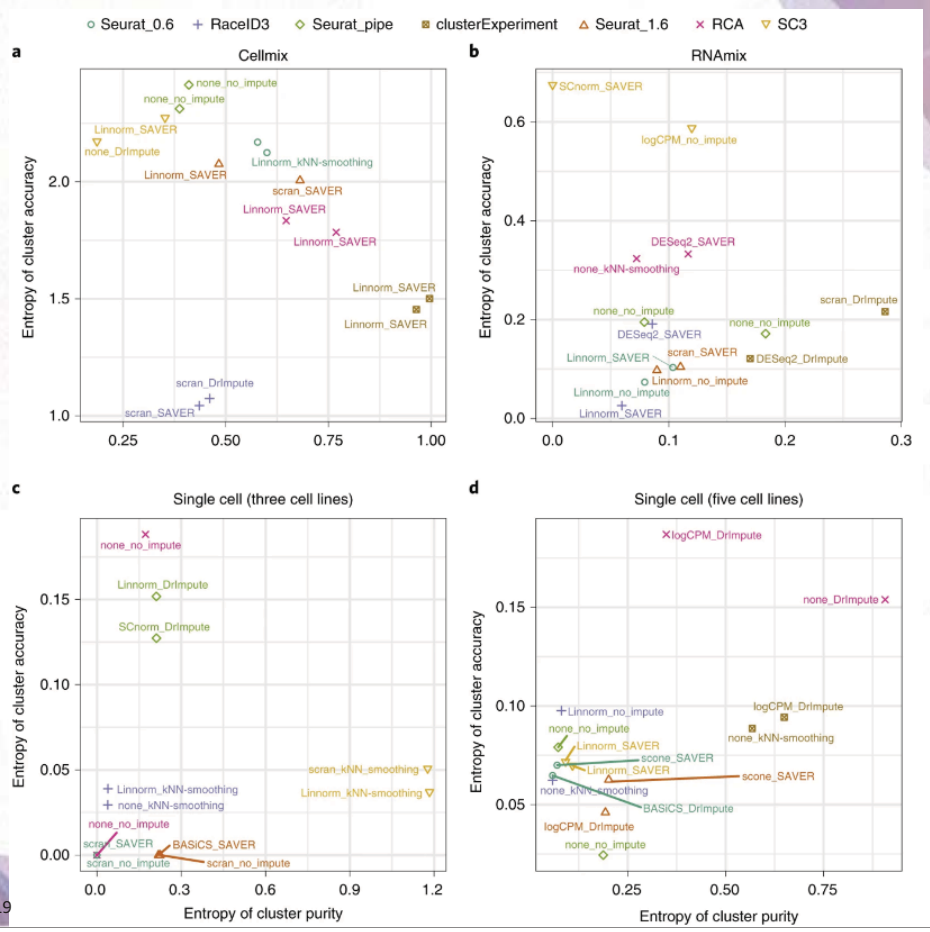
Name	Year	Method type	Strengths	Limitations
scanpy[4]	2018	PCA + graph-based	Very scalable	May not be accurate for small data sets
Seurat (latest)[3]	2016			
PhenoGraph[32]	2015			
SC3 (REF.[22])	2017	PCA + *k*-means	High accuracy through consensus, provides estimation of *k*	High complexity, not scalable
SIMLR[24]	2017	Data-driven dimensionality reduction + *k*-means	Concurrent training of the distance metric improves sensitivity in noisy data sets	Adjusting the distance metric to make cells fit the clusters may artificially inflate quality measures
CIDR[25]	2017	PCA + hierarchical	Implicitly imputes dropouts when calculating distances	
GiniClust[75]	2016	DBSCAN	Sensitive to rare cell types	Not effective for the detection of large clusters
pcaReduce[27]	2016	PCA + *k*-means + hierarchical	Provides hierarchy of solutions	Very stochastic, does not provide a stable result
Tasic et al.[28]	2016	PCA + hierarchical	Cross validation used to perform fuzzy clustering	High complexity, no software package available
TSCAN[41]	2016	PCA + Gaussian mixture model	Combines clustering and pseudotime analysis	Assumes clusters follow multivariate normal distribution
mpath[45]	2016	Hierarchical	Combines clustering and pseudotime analysis	Uses empirically defined thresholds and a priori knowledge
BackSPIN[26]	2015	Biclustering (hierarchical)	Multiple rounds of feature selection improve clustering resolution	Tends to over-partition the data
RaceID[23], RaceID2 (REF.[115]), RaceID3	2015	*k*-Means	Detects rare cell types, provides estimation of *k*	Performs poorly when there are no rare cell types
SINCERA[5]	2015	Hierarchical	Method is intuitively easy to understand	Simple hierarchical clustering is used, may not be appropriate for very noisy data
SNN-Cliq[80]	2015	Graph-based	Provides estimation of *k*	High complexity, not scalable

DBSCAN, density-based spatial clustering of applications with noise; PCA, principal component analysis; scRNA-seq, single-cell RNA sequencing.

Nature Reviews. 2019

42

Comparisons of scRNA-seq clustering methods

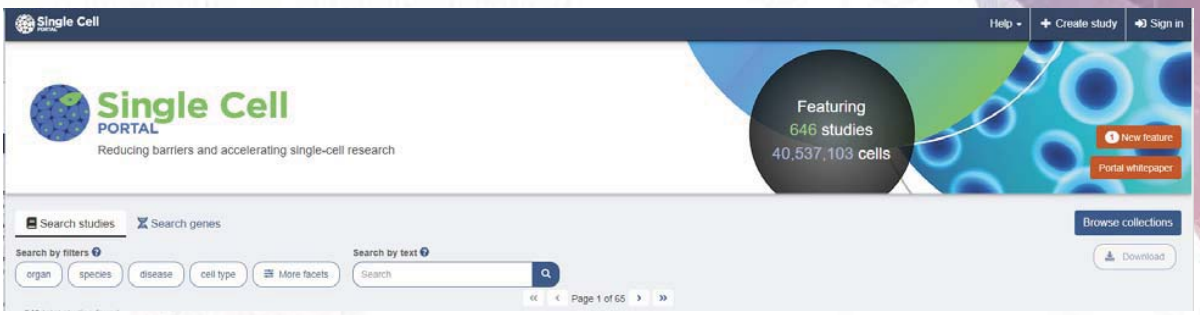


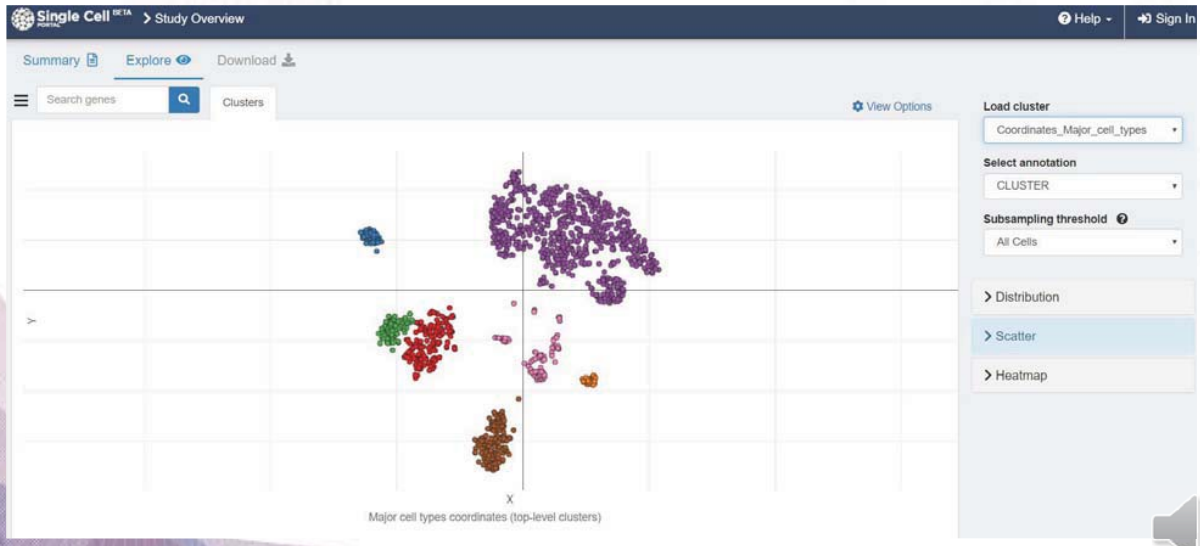
Nature Methods. 2019

43

Databases

44





https://portals.broadinstitute.org/single_cell

45

Data availability. All raw sequencing data are available in ArrayExpress under accessions E-MTAB-6149 and E-MTAB-6653. Also, Rds files were uploaded. These can be imported in CellView to visualise clusters, scroll through tSNE projections and explore gene expression. Moreover, scRNA-seq source data were formatted as .loom files, which can be visualized in an interactive manner through SCope (https://gbiomed.kuleuven.be/scRNAseq-NSCLC)[54]. Finally, gene expression data for all 52 clusters are available in Supplementary Table 4, and cluster-specific gene expression data for tumor-derived and non-malignant lung-tissue-derived cells are available in Supplementary Table 5 (only for clusters having >100 cells from both sources).

Nat Med. 2018

46

EMBL-EBI | Services | Research | Training | About us | EMBL-EBI Hinxton

ArrayExpress

Search

Examples: E-MEXP-31, cancer, p53, Geuvadis | advanced search

Home | Browse | Submit | Help | About ArrayExpress | Contact Us | Login

ArrayExpress – functional genomics data

ArrayExpress Archive of Functional Genomics Data stores data from high-throughput functional genomics experiments, and provides these data for reuse to the research community.

Browse ArrayExpress

Data Content

Updated today at 03:00

- 72158 experiments
- 2373008 assays
- 54.54 TB of archived data

https://www.ebi.ac.uk/arrayexpress/

E-MTAB-6149 - Single cell sequencing of lung carcinoma

E-MTAB-6149.processed.1.zip	6.7 MB	14 March 2018, 13:36
E-MTAB-6149.processed.2.zip	13.2 MB	14 March 2018, 13:36
E-MTAB-6149.processed.3.zip	157.6 MB	14 March 2018, 13:36
E-MTAB-6149.processed.4.zip	57.4 MB	14 March 2018, 13:36
E-MTAB-6149.processed.5.zip	40.4 MB	14 March 2018, 13:36
E-MTAB-6149.processed.6.zip	5.8 MB	14 March 2018, 13:36
E-MTAB-6149.processed.7.zip	7.6 MB	14 March 2018, 13:36
E-MTAB-6149.idf.txt	6 KB	9 July 2018, 16:39
E-MTAB-6149.sdrf.txt	66 KB	15 March 2018, 08:18
1247.R1.fastq.gz	7.94 GB	14 March 2018, 13:45
1247.R2.fastq.gz	1.72 GB	14 March 2018, 13:46
1247.R3.fastq.gz	5.35 GB	14 March 2018, 13:46
BT1249.R1.fastq.gz	5.47 GB	14 March 2018, 13:47
BT1249.R2.fastq.gz	1.14 GB	14 March 2018, 13:47
BT1249.R3.fastq.gz	3.69 GB	14 March 2018, 13:47
BT1290_R1.fastq.gz	1.48 GB	14 March 2018, 13:47
BT1290_R2.fastq.gz	2.94 GB	14 March 2018, 13:47
BT1291_R1.fastq.gz	6.92 GB	14 March 2018, 13:48
BT1291_R2.fastq.gz	14.61 GB	14 March 2018, 13:49

Processed data

Investigation description

Sample and data relationship

Raw data

47

Home | Search | Help | About | scRNASeqDB

scRNASeqDB

a database for gene expression profiling in human single cell by RNA-seq

Welcome to scRNASeqDB!

Single-cell RNA-Seq (scRNA-seq) are an emerging method which facilitates to explore the comprehensive transcriptome in a single cell. To provide a useful and unique reference resource for biology and medicine, we developed the scRNASeqDB database, which contains 36 human single cell gene expression data sets collected from Gene Expression Omnibus (GEO), involving 8910 cells from 174 cell groups. We also provides detailed information for gene expression of cells in different status, as well as some features, including heatmap and boxplot of gene expression, gene correlation matrix, GO and pathway annotations.

You can also submit scRNASeq data sets to our database. Feel free to contact us if you have any questions!

Current curation

Number of GSE datasets: 38

Number of GSM entries: 13440

Number of cell groups: 200

New datasets

GSE86982 REGION-SPECIFIC NEURAL STEM CELL LINEAGES REVEALED BY SINGLE-CELL RNA-SEQ FROM HUMAN EMBRYONIC STEM CELLS [Smart-seq]

GSE86977 REGION-SPECIFIC NEURAL STEM CELL LINEAGES REVEALED BY SINGLE-CELL RNA-SEQ FROM HUMAN EMBRYONIC STEM CELLS [Cel-seq]

GSE77564 Coupled electrophysiological recording and single-cell transcriptome analyses revealed molecular mechanisms underlying neuronal maturation

Publication

Yuan Cao, Junjie Zhu, Guangchun Han, Peilin Jia, Zhongming Zhao. scRNASeqDB: a database for gene expression profiling in human single cell by RNA-seq (in review). bioRxiv

Search scRNASeqDB

By Gene | By Cell

Gene symbol | Gene Ensembl ID

TBK1 Search

Please input gene symbol of Ensembl ID

Gene Cloud

RPS8 RPL19 RPS6 RPL8 CUEDC2 CD59 NDUFB2 UBB TPT1 TBK1 RPS12 RPLP1 ACTG1 EEF1A1 TMSB4X NPM1 MAP1B RPS14 GNAS HLA-E RPS24 PGAM1 ACTB RPL27 RNF34 RPS19 BAP1 HSPA8 FTL HINT1 B2M GAPDH SCG5 RPL6 RPL7 RPLP2

News

More

GSE86982 has been added to our database.	2017/03/31
GSE86977 has been added to our database.	2017/03/29
CIDR has been used to cluster single cells in each dataset.	2017/03/12
Rankprod has been used to rank gene expression across all datasets.	2017/03/03
scRNASeqDB has been launched.	2016/09/15

48

Journal papers on scRNA-seq analysis of cancer

49

nature medicine

RESOURCE

https://doi.org/10.1038/s41591-018-0096-5

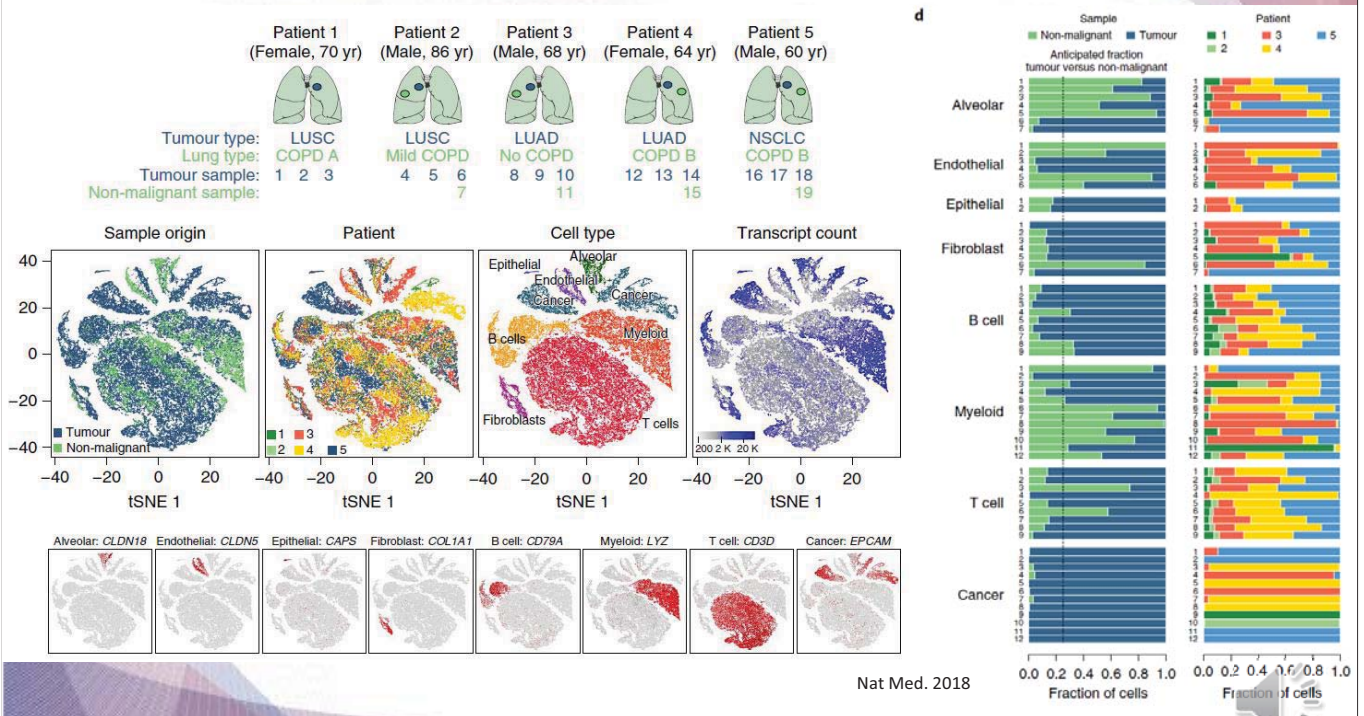
Phenotype molding of stromal cells in the lung tumor microenvironment

Diether Lambrechts[1,2*], Els Wauters[3,4], Bram Boeckx[1,2], Sara Aibar[5,6], David Nittner[7,8], Oliver Burton[6,9], Ayse Bassez[1,2], Herbert Decaluwé[10,11], Andreas Pircher[1,12], Kathleen Van den Eynde[13], Birgit Weynand[13], Erik Verbeken[13], Paul De Leyn[11], Adrian Liston[6,9], Johan Vansteenkiste[3,4], Peter Carmeliet[1,12,14], Stein Aerts[5,6] and Bernard Thienpont[1,15*]

Cancer cells are embedded in the tumor microenvironment (TME), a complex ecosystem of stromal cells. Here, we present a 52,698-cell catalog of the TME transcriptome in human lung tumors at single-cell resolution, validated in independent samples where 40,250 additional cells were sequenced. By comparing with matching non-malignant lung samples, we reveal a highly complex TME that profoundly molds stromal cells. We identify 52 stromal cell subtypes, including novel subpopulations in cell types hitherto considered to be homogeneous, as well as transcription factors underlying their heterogeneity. For instance, we discover fibroblasts expressing different collagen sets, endothelial cells downregulating immune cell homing and genes coregulated with established immune checkpoint transcripts and correlating with T-cell activity. By assessing marker genes for these cell subtypes in bulk RNA-sequencing data from 1,572 patients, we illustrate how these correlate with survival, while immunohistochemistry for selected markers validates them as separate cellular entities in an independent series of lung tumors. Hence, in providing a comprehensive catalog of stromal cells types and by characterizing their phenotype and co-optive behavior, this resource provides deeper insights into lung cancer biology that will be helpful in advancing lung cancer diagnosis and therapy.

50

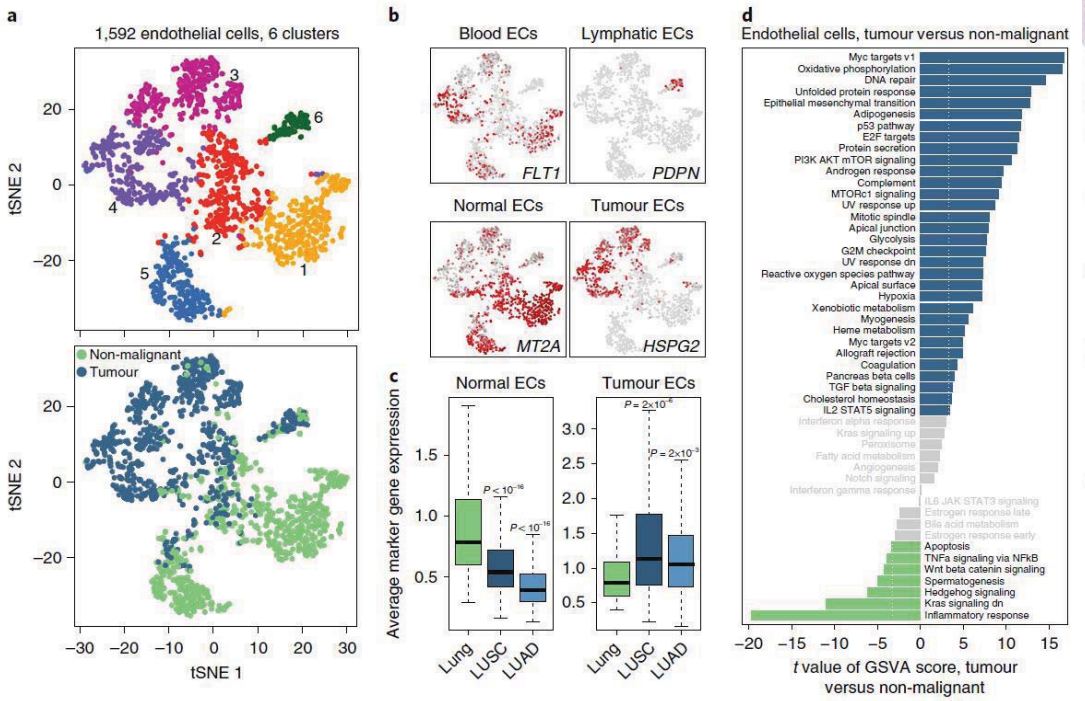
Many stromal cell subclusters were enriched for either tumor-derived or lung tissue-derived cells



Nat Med. 2018

51

Myc targets as the top enriched signature in tumor endothelial cells

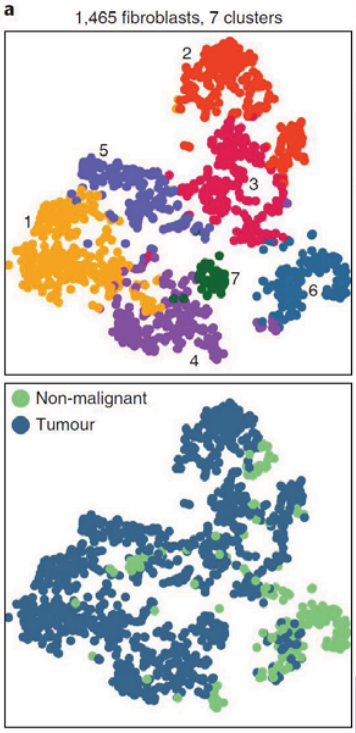


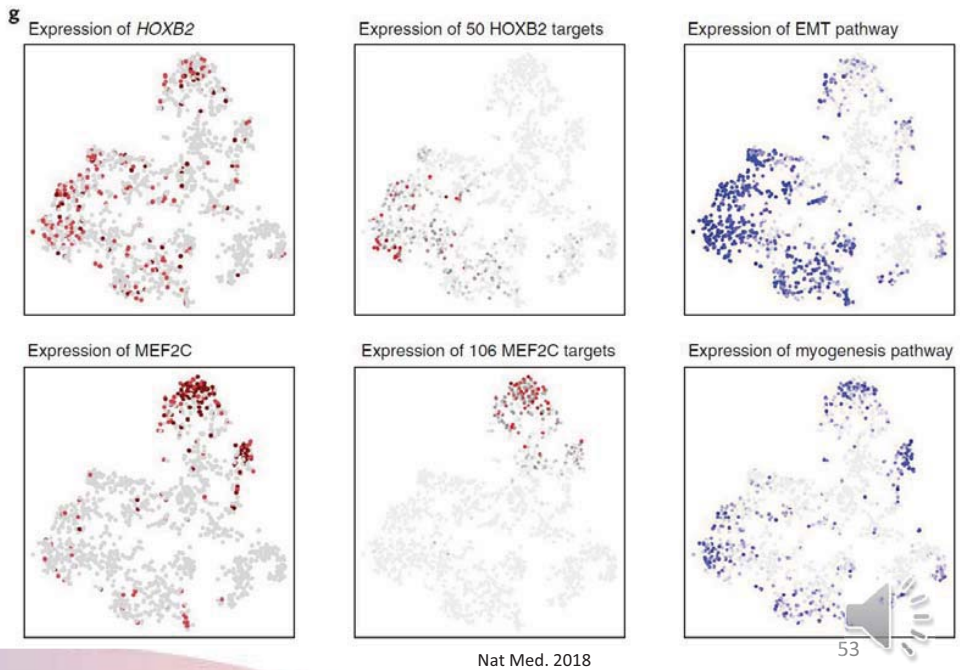
Nat Med. 2018

- Most significantly downregulated pathway was involved in inflammatory responses.

52

Lung tumors are enriched with fibroblasts with expression of EMT pathway





Nat Med. 2018

53

Preparation of single-cell suspensions. Following resection in the operating room, samples from the tumor and adjacent non-malignant lung tissue from the same resection specimen at maximal distance (>5 cm) from the tumor were isolated and transported rapidly to the research facility. On arrival, samples were rinsed with PBS and the tumor sample macroscopically examined for tumor positioning. The tumor sample was subsequently divided into three pieces, with one piece containing mainly tissue derived from the tumor core, one piece containing tissue mainly derived from the tumor edge and a third piece originating from the position intermediate to the other two samples. Each sample was subsequently minced on ice to smaller pieces of less than 1 mm^3 and transferred to 10 ml digestion medium containing 0.2% collagenase I/II (ThermoFisher Scientific), DNAse I (Sigma) and 25 units dispase (Invitrogen) in DMEM (ThermoFisher Scientific). Samples were incubated for 15 min at 37 °C, with manual shaking every 5 min. Samples were then vortexed for 10 s and pipetted up and down for 1 min using pipettes of descending sizes (25 ml, 10 ml and 5 ml). Next, 30 ml ice-cold PBS, pH 7.4, (ThermoFisher Scientific) containing 2% fetal bovine serum (ThermoFisher Scientific) was added and samples were filtered using a 40-µm nylon mesh (ThermoFisher Scientific). Following centrifugation at 120× *g* and 4 °C for 5 min, the supernatant was decanted and discarded, and the cell pellet was resuspended in 2 ml red blood cell lysis buffer and transferred to a 2-ml DNA low bind tube. Following a 5-min incubation at room temperature, samples were centrifuged (120×*g*, 4 °C, 5 min) using a swing-out rotor. Samples were next resuspended in 1 ml PBS containing 8 µl UltraPure BSA (50 mg ml^{-1}; AM2616, ThermoFisher Scientific) and filtered over Scienceware Flowmi 40-µm cell strainers (VWR) using wide-bore 1 ml low-retention filter tips (Mettler-Toledo). Next, 10 µl of this cell suspension was counted using an automated cell counter (Luna) to determine the concentration of live cells. Throughout the dissociation procedure, cells were maintained on ice whenever possible, and the entire procedure was completed in less than 1 h (typically ~45 min) to avoid dissociation-associated artefacts recently described[13]. By using a dissociation signature[13] to detect dissociation-associated changes in gene expression, a positive signal for less than 2% of cells was detected (Supplementary Fig. 2a).

Nat Med. 2018

54

Droplet-based scRNA-seq. Single-cell suspensions were converted to barcoded scRNA-seq libraries by using the Chromium Single Cell 3' Library, Gel Bead & Multiplex Kit and Chip Kit (10x Genomics), aiming for an estimated 4,000 cells per library and following the manufacturer's instructions. Samples were processed using kits pertaining to either the V1 or V2 barcoding chemistry of 10x Genomics (Supplementary Table 2). Single samples are always processed in a single well of a PCR plate, allowing all cells from a sample to be treated with the same master mix and in the same reaction vessel. For each patient, all samples (non-malignant and tumor) were processed in parallel in the same thermal cycler. Libraries were sequenced on an Illumina HiSeq4000, and mapped to the human genome (build hg19) using CellRanger (10x Genomics). Gene positions were annotated as per Ensembl build 85 and filtered for biotype (only protein-coding, long intergenic non-coding RNA, antisense, immunoglobulin or T-cell receptor).

Nat Med. 2018

55

Single-cell gene expression quantification and determination of the major cell types. Raw gene expression matrices generated per sample using CellRanger (version 2.0.0) were combined in R (version 3.3.2—*Sincere Pumpkin Patch*), and converted to a Seurat object using the Seurat R package (version 1.4.0.7)[11]. From this, all cells were removed that had either fewer than 201 UMIs, over 6,000 or below 101 expressed genes, or over 10% UMIs derived from mitochondrial genome. From the remaining 52,698 cells, gene expression matrices were normalized to total cellular read count and to mitochondrial read count using linear regression as implemented in Seurat's *RegressOut* function. As a result, none of the principle components subsequently identified were correlated with transcript count (data not shown). From the remaining 52,698 cells, variably expressed genes were selected as having a normalized expression between 0.125 and 3, and a quantile-normalized variance exceeding 0.5. To reduce dimensionality of this dataset, the resulting 2,192 variably expressed genes were summarized by principle component analysis, and the first 8 principle components further summarized using tSNE dimensionality reduction using the default settings of the *RunTSNE* function. Cell clusters in the resulting two-dimensional representation were annotated to known biological cell types using canonical marker genes (Supplementary Fig. 1). Of note, very few stromal cells (~2%) were positive for cell proliferation markers (Supplementary Fig. 4). We therefore opted not to correct our gene expression matrices for effects of cell cycle.

Nat Med. 2018

56

Subclustering of the major cell types. To identify subclusters within these eight cell types, we reanalyzed cells belonging to each of these eight cell types separately. Specifically, we applied dimensionality reduction using principle component analysis in each cell type on variably expressed genes as described above. To identify which principle components were informative, we applied Horn's parallel analysis for principle component analysis[44] as implemented in the R *paran* package (version 1.5.1.), selecting those principle components having eigenvalues that exceed the eigenvalues generated using ten random permutations by >50%. Using the graph-based clustering approach implemented in the *FindClusters* function of the Seurat package, with a conservative resolution of 0.5 and otherwise default parameters, each cell type was reclustered by its principle components. Notably, subclustering was robust to alterations in the number of principle components, in the resolution or in the *K* parameter (Supplementary Fig. 3a–c). Moreover, few of the subclusters identified contained many cells wherein less than 300 genes were detected, indicating that increasing the threshold of 100 genes will not affect our results (Supplementary Fig. 20). This yielded 64 subclusters (52 stromal subclusters) in total, as listed in Supplementary Table 3. For visualization purposes, these informative principle components were converted into tSNE plots as above.

Nat Med. 2018

57

Identification of marker genes. To identify marker genes for each of these 64 subclusters within these 8 cell types, we contrasted cells from that subcluster to all other cells of that subcluster using the Seurat *FindMarkers* function. Marker genes were required to have an average expression in that subcluster that was >2.5-fold higher than the average expression in the other subclusters from that cell type, and a detectable expression in >15% of all cells from that subcluster. Additionally, marker genes were required to have the highest mean expression in that subcluster, out of all 64 subclusters. This yielded a list of in total 402 marker genes (Supplementary Table 3) for 51 subclusters (42 stromal cell subclusters), whereas for 13 subclusters we failed to identify marker genes. When analyzing marker genes for several subclusters in aggregate, such as for tumor endothelial cells (endothelial cell clusters 3 and 4) or for macrophages (myeloid clusters 1–4, 6–8, 10 and 11), we simply combined the marker genes for all associated subclusters.

Nat Med. 2018

58

nature medicine

LETTERS

https://doi.org/10.1038/s41591-018-0045-3

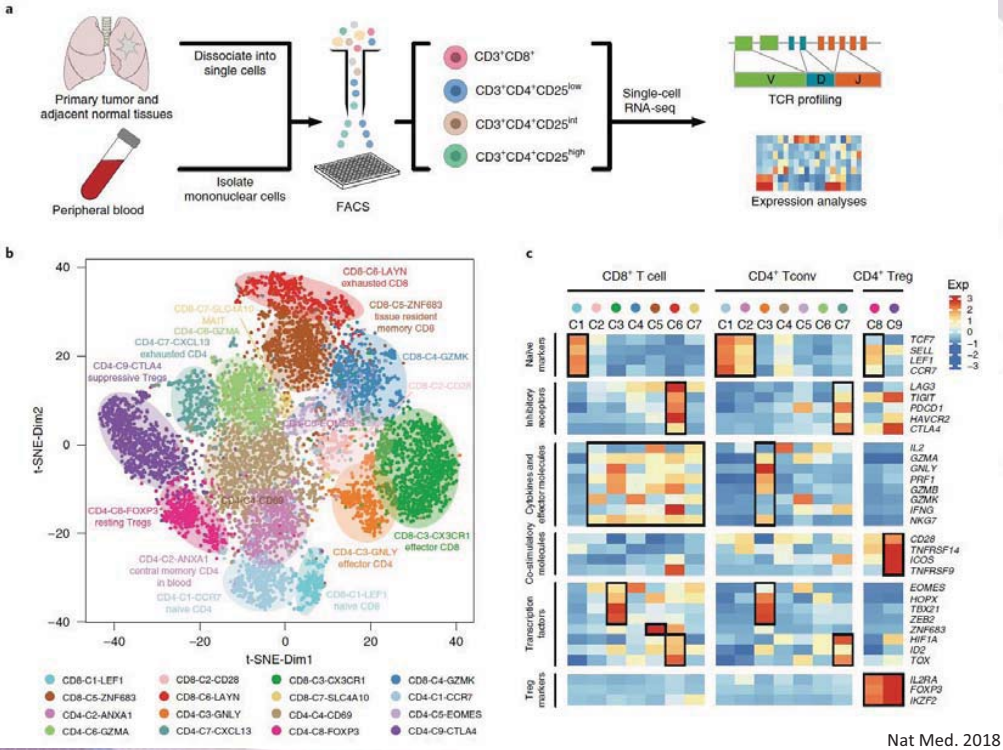
Global characterization of T cells in non-small-cell lung cancer by single-cell sequencing

Xinyi Guo[1,6], Yuanyuan Zhang[1,6], Liangtao Zheng[2,6], Chunhong Zheng[1,6], Jintao Song[3,6], Qiming Zhang[1], Boxi Kang[1], Zhouzerui Liu[1], Liang Jin[3], Rui Xing[4], Ranran Gao[1], Lei Zhang[2], Minghui Dong[1], Xueda Hu[1], Xianwen Ren[1], Dennis Kirchhoff[5], Helge Gottfried Roider[5], Tiansheng Yan[3]* and Zemin Zhang[1,2]*

Cancer immunotherapies have shown sustained clinical responses in treating non-small-cell lung cancer[1-3], but efficacy varies and depends in part on the amount and properties of tumor infiltrating lymphocytes[4-6]. To depict the baseline landscape of the composition, lineage and functional states of tumor infiltrating lymphocytes, here we performed deep single-cell RNA sequencing for 12,346 T cells from 14 treatment-naïve non-small-cell lung cancer patients. Combined expression and T cell antigen receptor based lineage tracking revealed a significant proportion of inter-tissue effector T cells with a highly migratory nature. As well as tumor-infiltrating CD8$^+$ T cells undergoing exhaustion, we observed two clusters of cells exhibiting states preceding exhaustion, and a high ratio of "pre-exhausted" to exhausted T cells was associated with better prognosis of lung adenocarcinoma. Additionally, we observed further heterogeneity within the tumor regulatory T cells (Tregs), characterized by the bimodal distribution of *TNFRSF9*, an activation marker for antigen-specific Tregs. The gene signature of those activated tumor Tregs, which included *IL1R2*, correlated with poor prognosis in lung adenocarcinoma. Our study provides a new approach for patient stratification and will help further understand the functional states and dynamics of T cells in lung cancer.

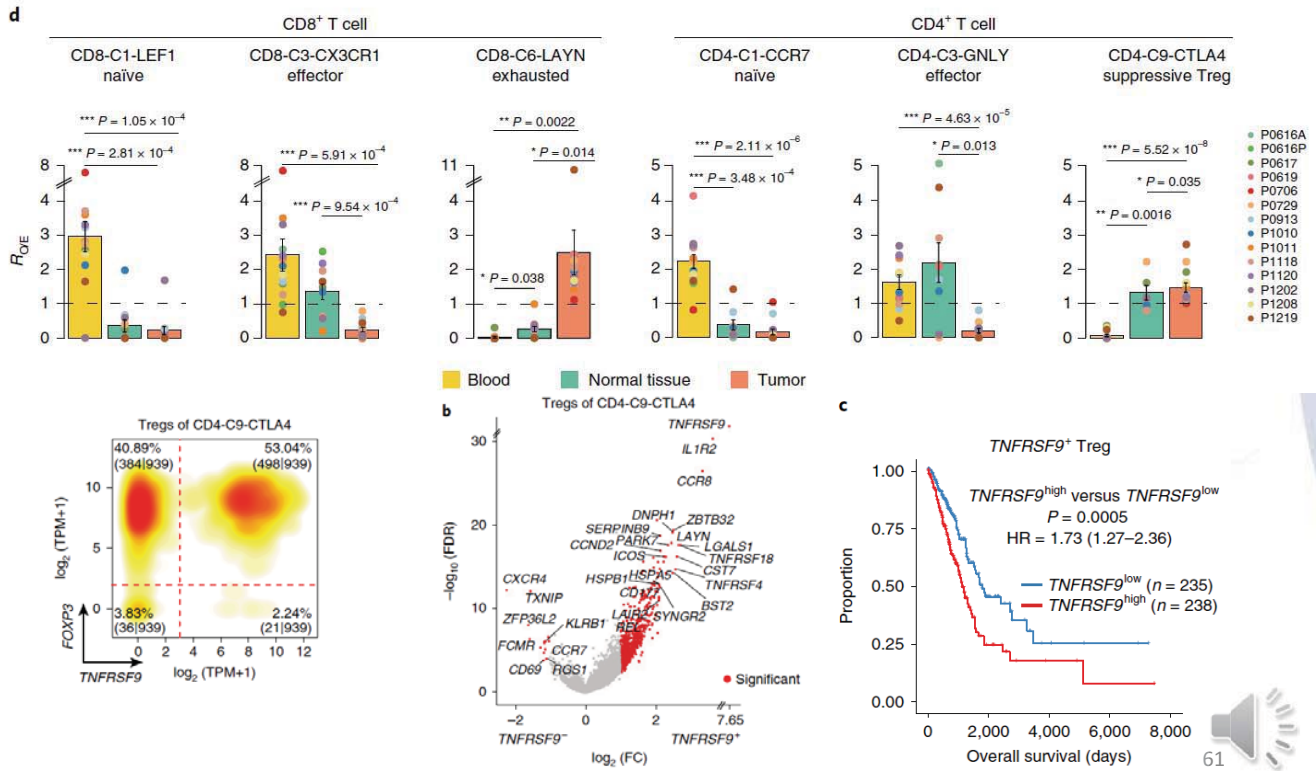
NATURE MEDICINE | VOL 24 | JULY 2018 | 978–985 | www.nature.com/naturemedicine

Seven CD8 and nine CD4 clusters were identified



Nat Med. 2018

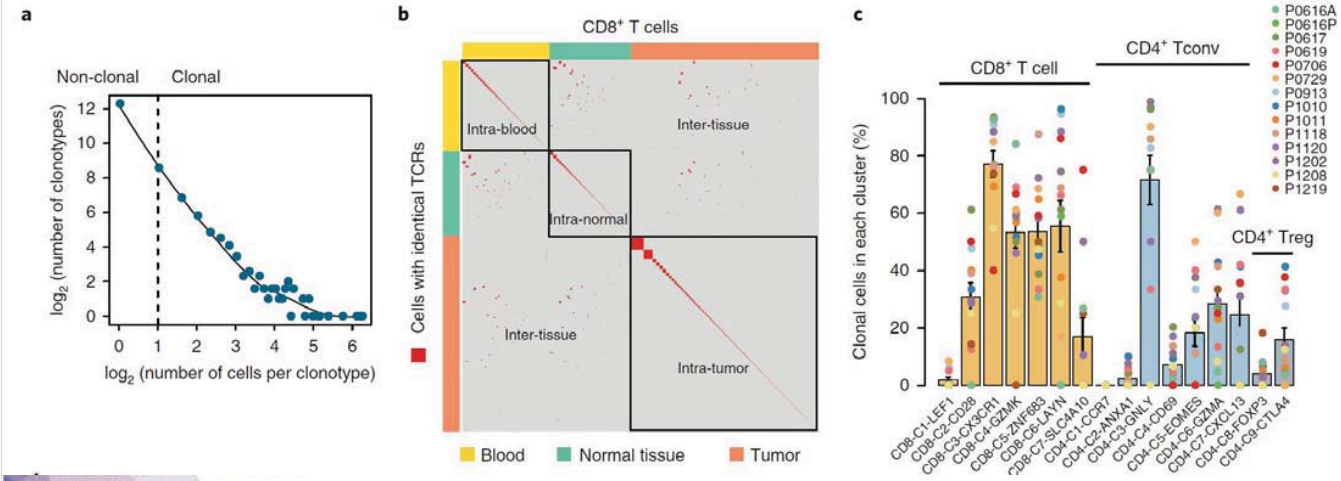
T cells clustered primarily based on their tissue origins and subtypes



61

Nat Med. 2018

T cell clusters CD8-C3-CX3CR1 and CD4-C3-GNLY showed the highest proportions of clonal cells



Nat Med. 2018

62

nature medicine

ARTICLES

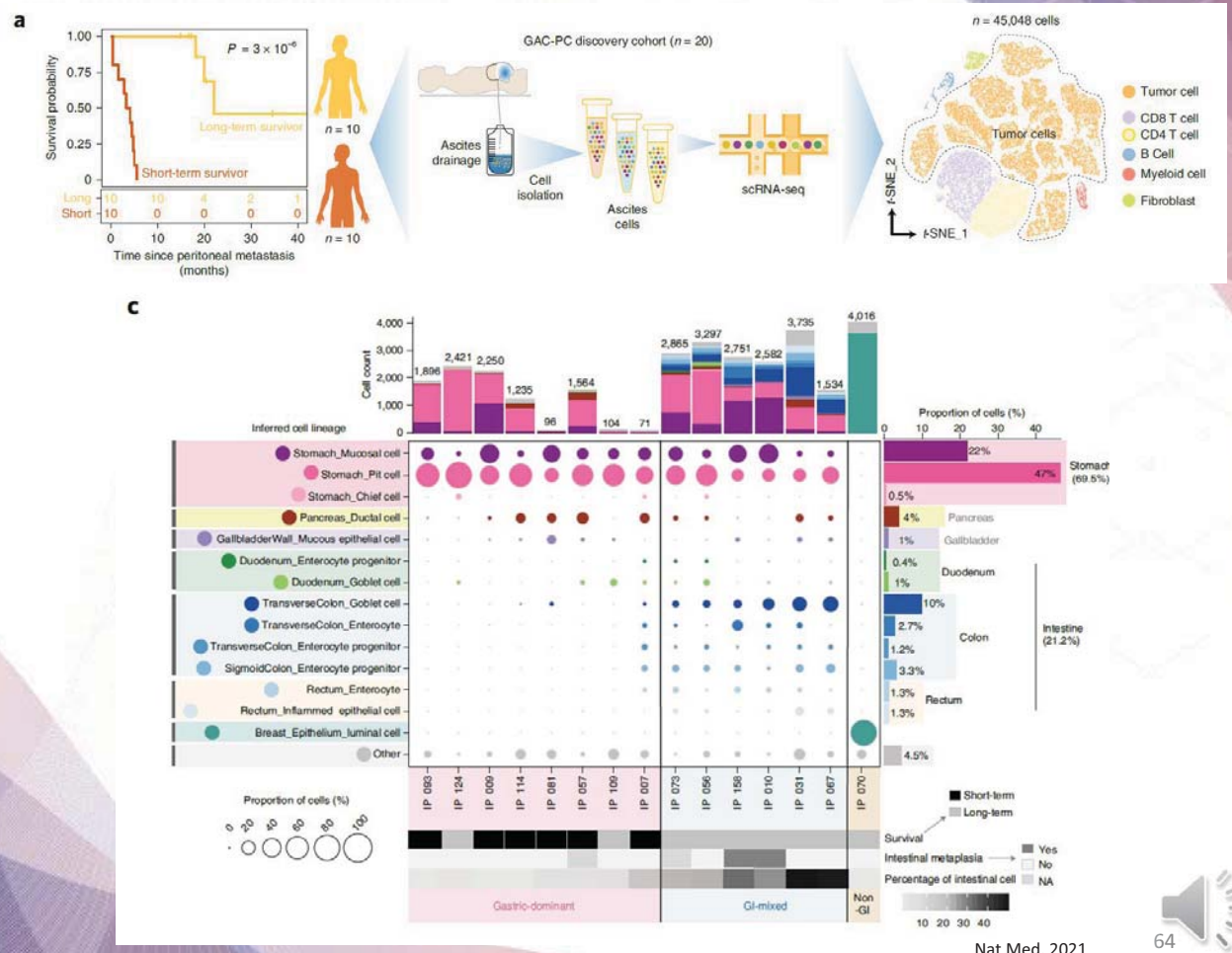
https://doi.org/10.1038/s41591-020-1125-8

Check for updates

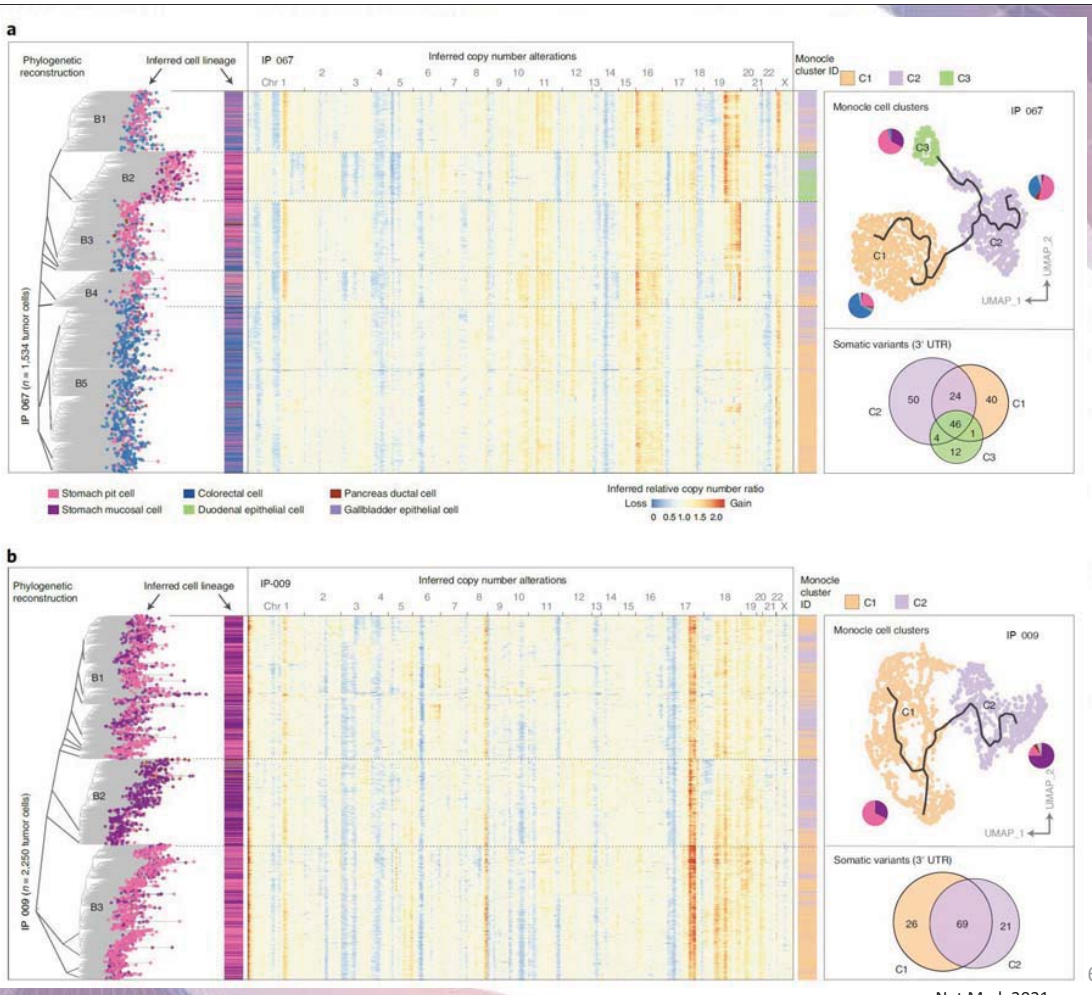
Single-cell dissection of intratumoral heterogeneity and lineage diversity in metastatic gastric adenocarcinoma

Ruiping Wang[1], Minghao Dang[1], Kazuto Harada[2,12], Guangchun Han[1], Fang Wang[3], Melissa Pool Pizzi[2], Meina Zhao[2], Ghia Tatlonghari[2], Shaojun Zhang[1], Dapeng Hao[1], Yang Lu[4], Shuangtao Zhao[1], Brian D. Badgwell[5], Mariela Blum Murphy[2], Namita Shanbhag[2], Jeannelyn S. Estrella[6], Sinchita Roy-Chowdhuri[6], Ahmed Adel Fouad Abdelhakeem[2], Yuanxin Wang[1], Guang Peng[7], Samir Hanash[7], George A. Calin[8], Xingzhi Song[1], Yanshuo Chu[1], Jianhua Zhang[1], Mingyao Li[9], Ken Chen[3], Alexander J. Lazar[6,10], Andrew Futreal[1], Shumei Song[2], Jaffer A. Ajani[2] ✉ and Linghua Wang[1,11] ✉

Intratumoral heterogeneity (ITH) is a fundamental property of cancer; however, the origins of ITH remain poorly understood. We performed single-cell transcriptome profiling of peritoneal carcinomatosis (PC) from 15 patients with gastric adenocarcinoma (GAC), constructed a map of 45,048 PC cells, profiled the transcriptome states of tumor cell populations, incisively explored ITH of malignant PC cells and identified significant correlates with patient survival. The links between tumor cell lineage/state compositions and ITH were illustrated at transcriptomic, genotypic, molecular and phenotypic levels. We uncovered the diversity in tumor cell lineage/state compositions in PC specimens and defined it as a key contributor to ITH. Single-cell analysis of ITH classified PC specimens into two subtypes that were prognostically independent of clinical variables, and a 12-gene prognostic signature was derived and validated in multiple large-scale GAC cohorts. The prognostic signature appears fundamental to GAC carcinogenesis and progression and could be practical for patient stratification.



Nat Med. 2021



Nat Med. 2021

65

Thank you!

Contact:

Semin Lee

Email: seminlee@unist.ac.kr

66

KSBI | 한국생명정보학회

KSBi-BIML 2024

Single-cell RNA-sequencing analysis of cancer

Hyoung-oh Jeong

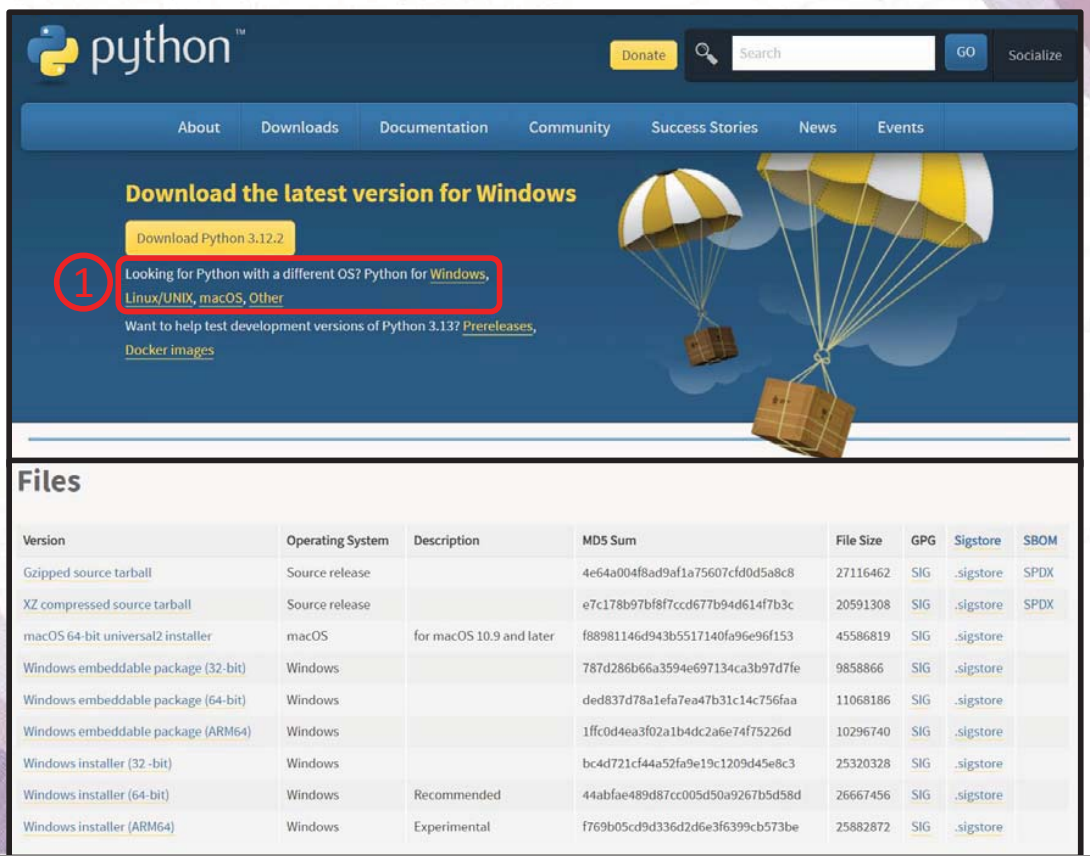
Email : hyoung-oh@unist.ac.kr

Ulsan National Institute of Science and Technology

Contents

1. Identify doublets (Scrublet)
2. Remove cell free mRNA contamination (SoupX)
3. single-cell RNA-seq analysis (Seurat)
4. Cell type annotation (SingleR)
5. Data Integration (Seuart)
6. Inferring copy number alterations (InferCNV)
7. Trajectory analysis (monocle)

Python 설치 (https://www.python.org/downloads/)



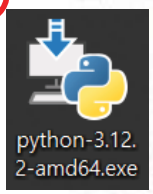
Files

Version	Operating System	Description	MD5 Sum	File Size	GPG	Sigstore	SBOM
Gzipped source tarball	Source release		4e64a004f8ad9af1a75607cfd0d5a8c8	27116462	SIG	.sigstore	SPDX
XZ compressed source tarball	Source release		e7c178b97bf8f7ccd677b94d614f7b3c	20591308	SIG	.sigstore	SPDX
macOS 64-bit universal2 installer	macOS	for macOS 10.9 and later	f88981146d943b5517140fa96e96f153	45586819	SIG	.sigstore	
Windows embeddable package (32-bit)	Windows		787d286b66a3594e697134ca3b97d7fe	9858866	SIG	.sigstore	
Windows embeddable package (64-bit)	Windows		ded837d78a1efa7ea47b31c14c756faa	11068186	SIG	.sigstore	
Windows embeddable package (ARM64)	Windows		1ffc0d4ea3f02a1b4dc2a6e74f75226d	10296740	SIG	.sigstore	
Windows installer (32-bit)	Windows		bc4d721cf44a52fa9e19c1209d45e8c3	25320328	SIG	.sigstore	
Windows installer (64-bit)	Windows	Recommended	44abfae489d87cc005d50a9267b5d58d	26667456	SIG	.sigstore	
Windows installer (ARM64)	Windows	Experimental	f769b05cd9d336d2d6e3f6399cb573be	25882872	SIG	.sigstore	

3

Python 설치

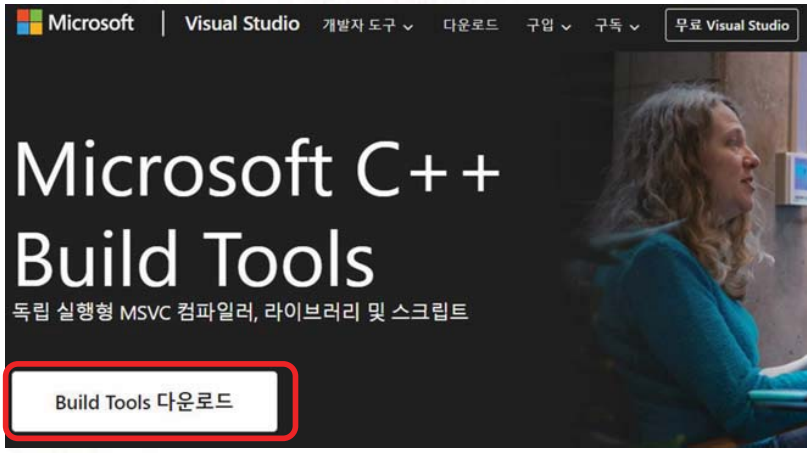
②



4

Python 설치

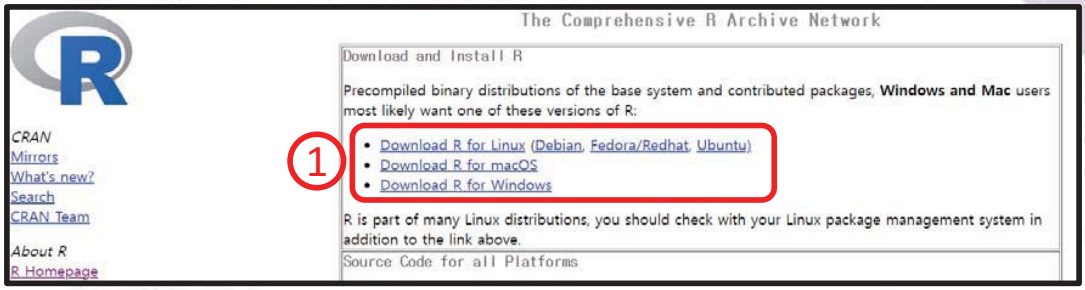
https://visualstudio.microsoft.com/ko/visual-cpp-build-tools/

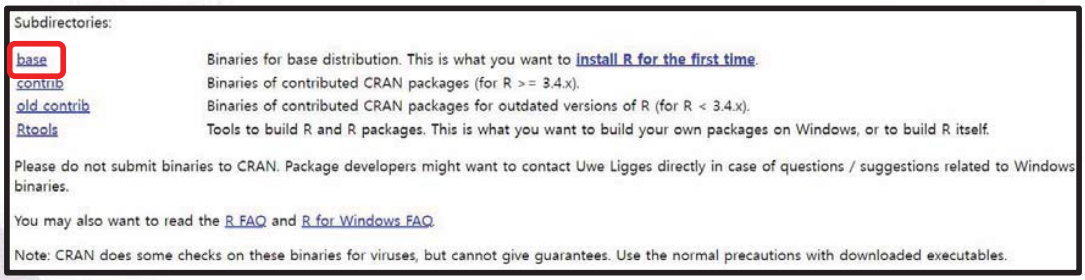


Python 설치

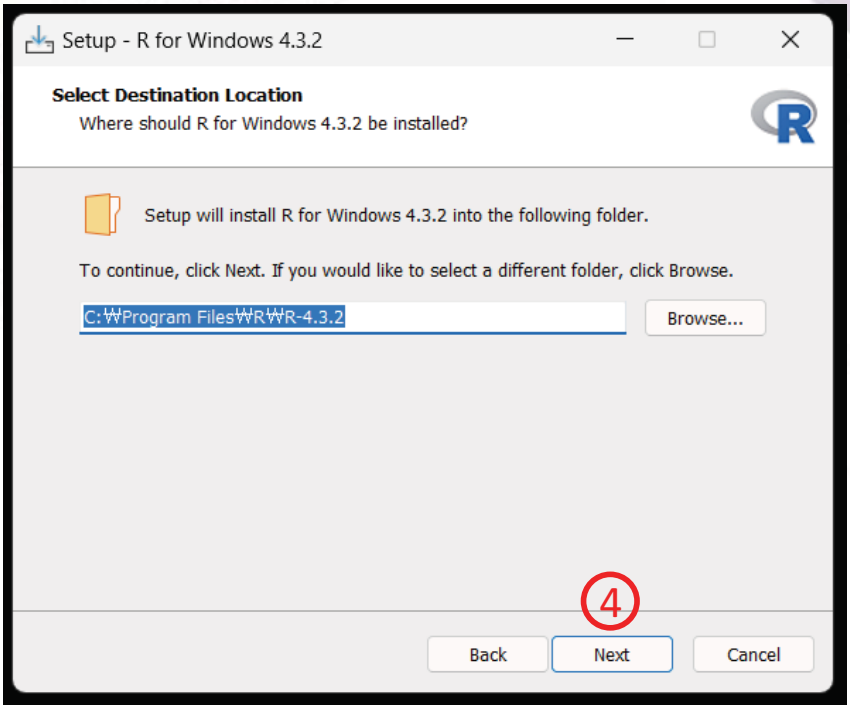
⑤

R 설치 (https://cran.yu.ac.kr/)





R 설치



RStudio 설치 (https://posit.co/download/rstudio-desktop/)

posit PRODUCTS SOLUTIONS LEARN & SUPPORT EXPLORE MORE PRICING

DOWNLOAD

RStudio Desktop

Used by millions of people weekly, the RStudio integrated development environment (IDE) is a set of tools built to help you be more productive with R and Python.

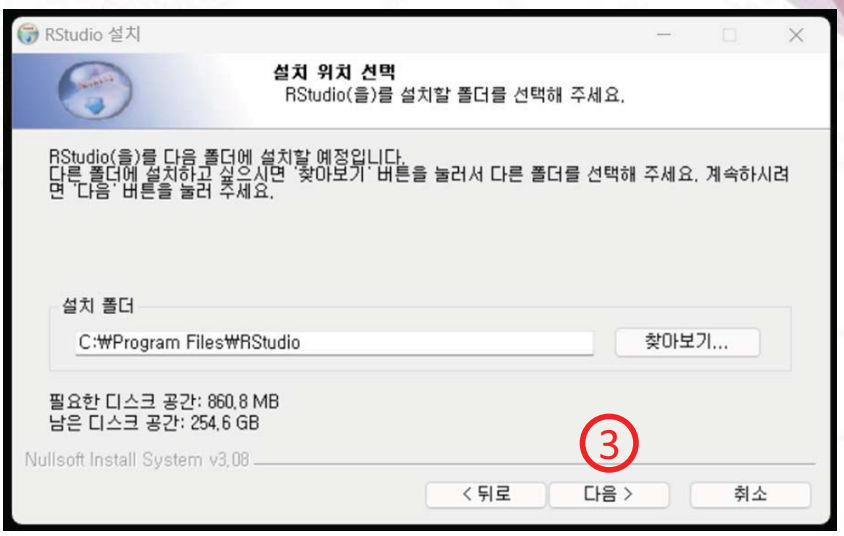
Don't want to download or install anything? Get started with RStudio on Posit Cloud for free. If you're a professional data scientist looking to download RStudio and also need common enterprise features, don't hesitate to book a call with us.

①

OS	Download	Size	SHA-256
Windows 10/11	RSTUDIO-2023.12.1-402.EXE	215.66 MB	D3C03C42
macOS 12+	RSTUDIO-2023.12.1-402.DMG	382.66 MB	C8D9185D
Ubuntu 20/Debian 11	RSTUDIO-2023.12.1-402-AMD64.DEB	149.27 MB	81F221BE
Ubuntu 22/Debian 12	RSTUDIO-2023.12.1-402-AMD64.DEB	149.96 MB	75542CC2

RStudio 설치

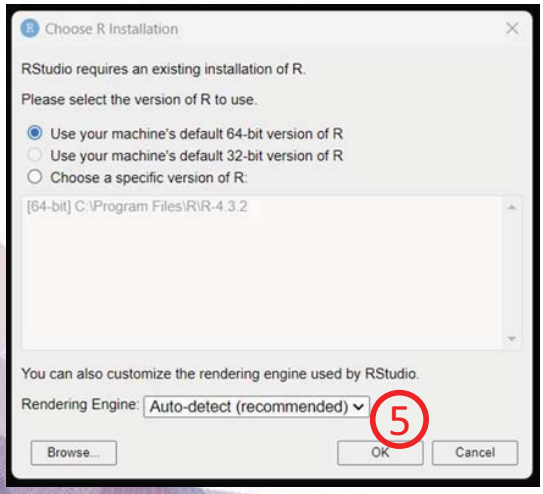
②



③

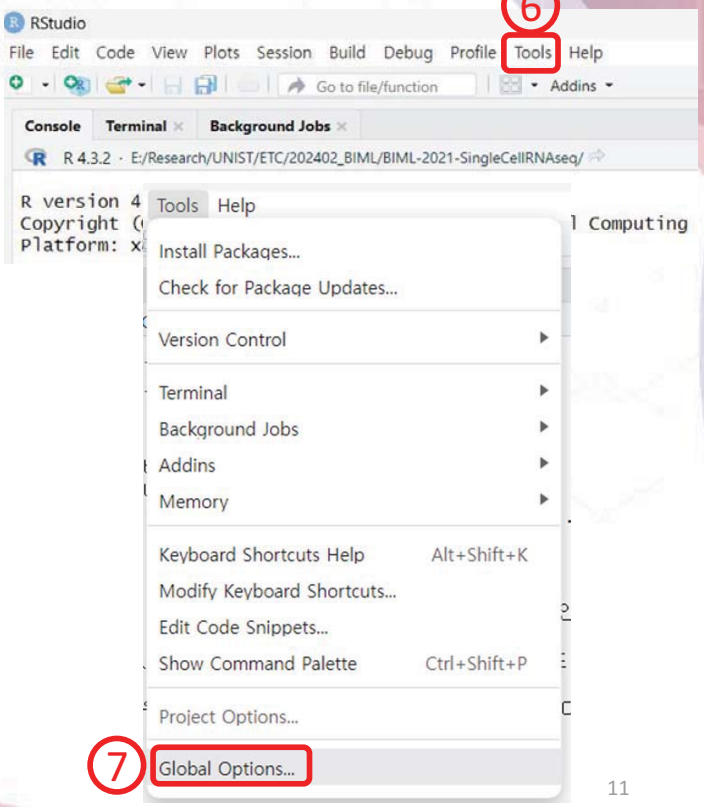
RStudio 설치

④



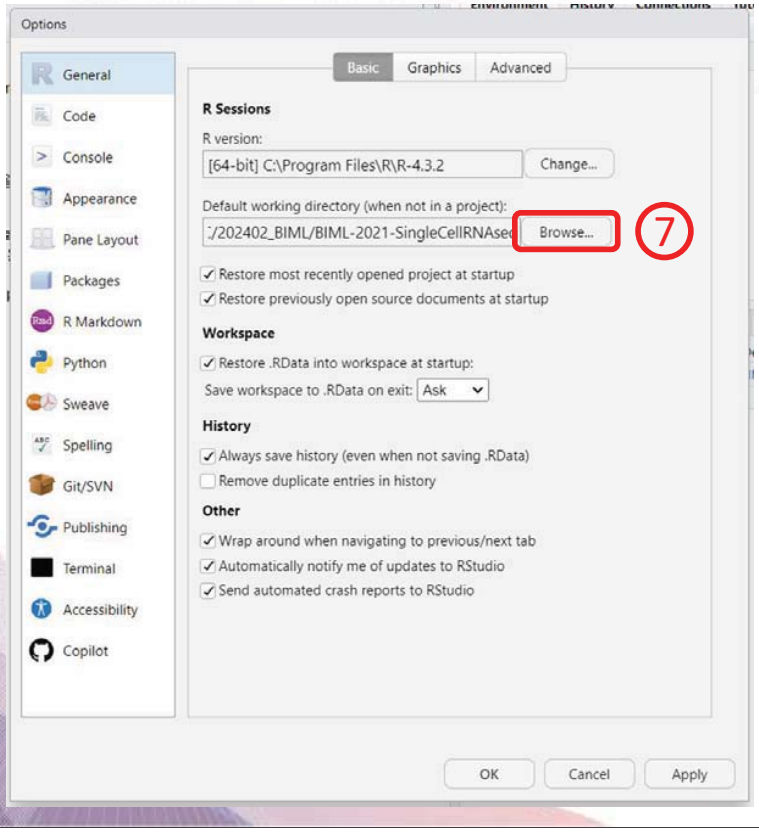
⑤

⑥



⑦

RStudio 설치 (https://posit.co/download/rstudio-desktop/)

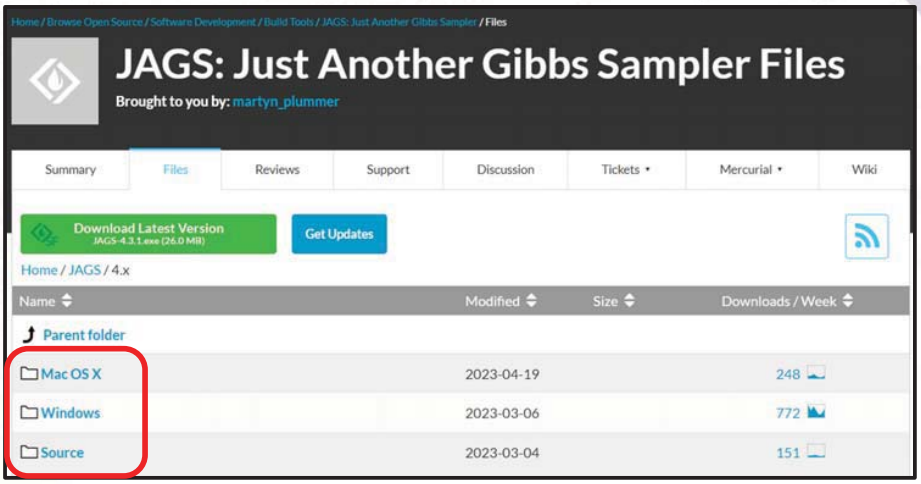


⑦

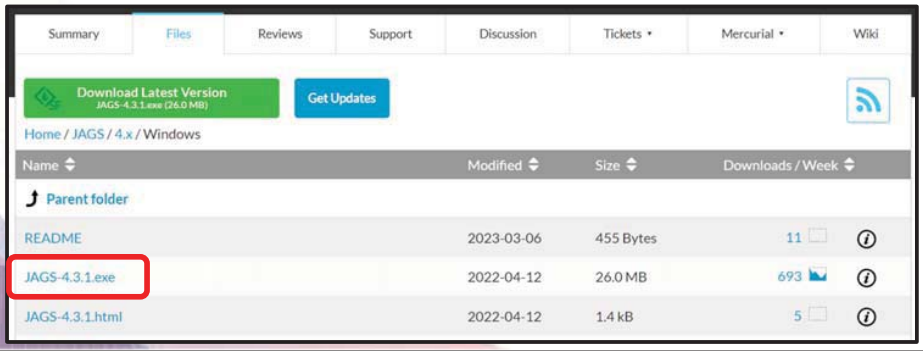
실습 자료가 있는 디렉토리 선택

* 이후 실습에서 "~/XXX"는 실습 자료가 있는 디렉토리를 의미합니다.

JAGS 설치 (https://sourceforge.net/projects/mcmc-jags/files/JAGS/4.x/)



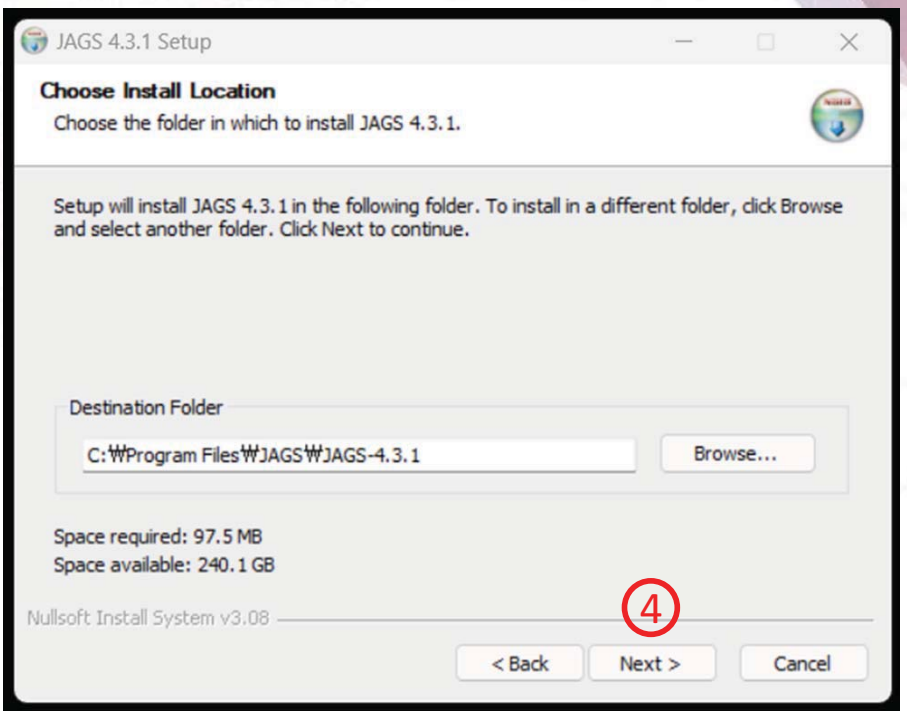
①



②

JAGS 설치

③

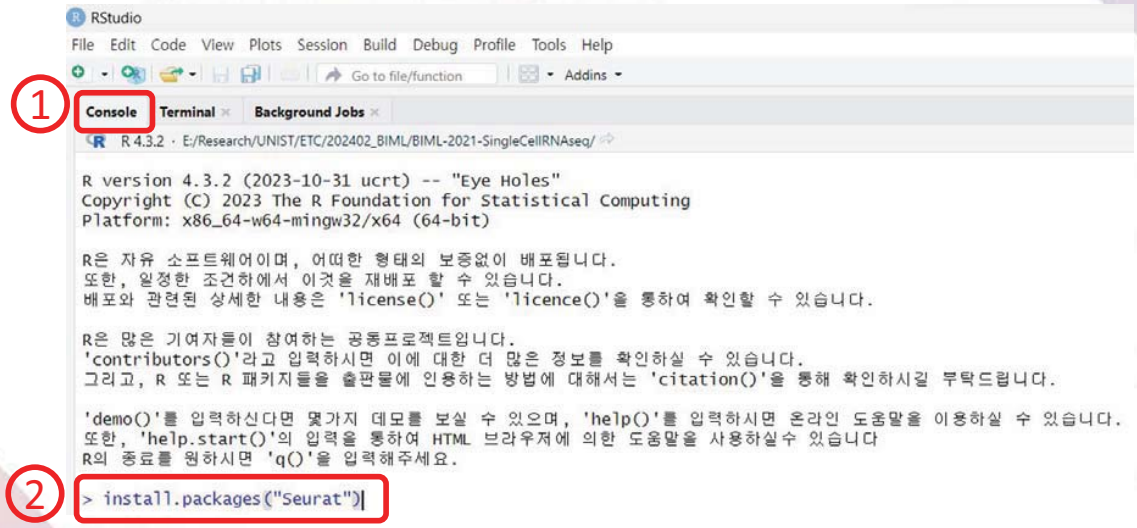


④

Python package 설치

- pip install scrublet

R package 설치



install.packages("Seurat")

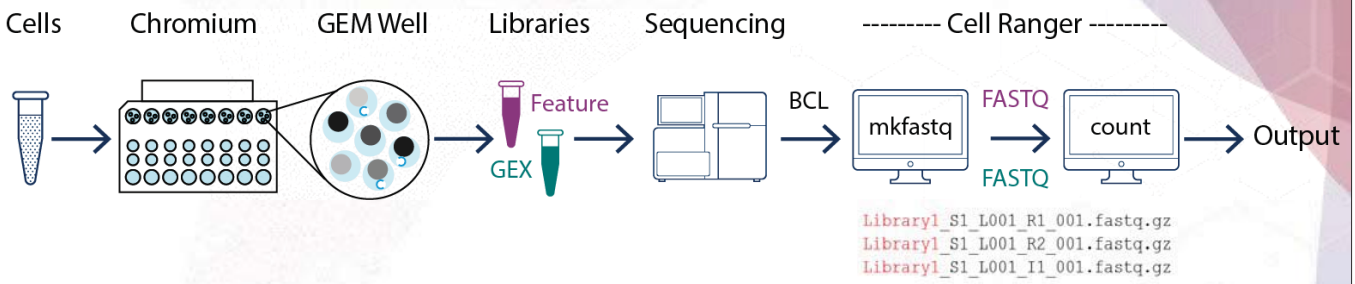
R package 설치

- if (!require("BiocManager", quietly = TRUE))
  install.packages("BiocManager")
- install.packages("devtools")

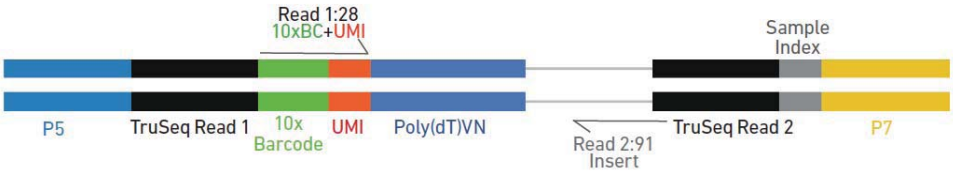
- install.packages("SoupX")
- BiocManager::install("DropletUtils")
- BiocManager::install("scater")
- BiocManager::install("SingleR")
- BiocManager::install("celldex")
- BiocManager::install("infercnv")
- BiocManager::install("monocle")

17

Generating FASTQ



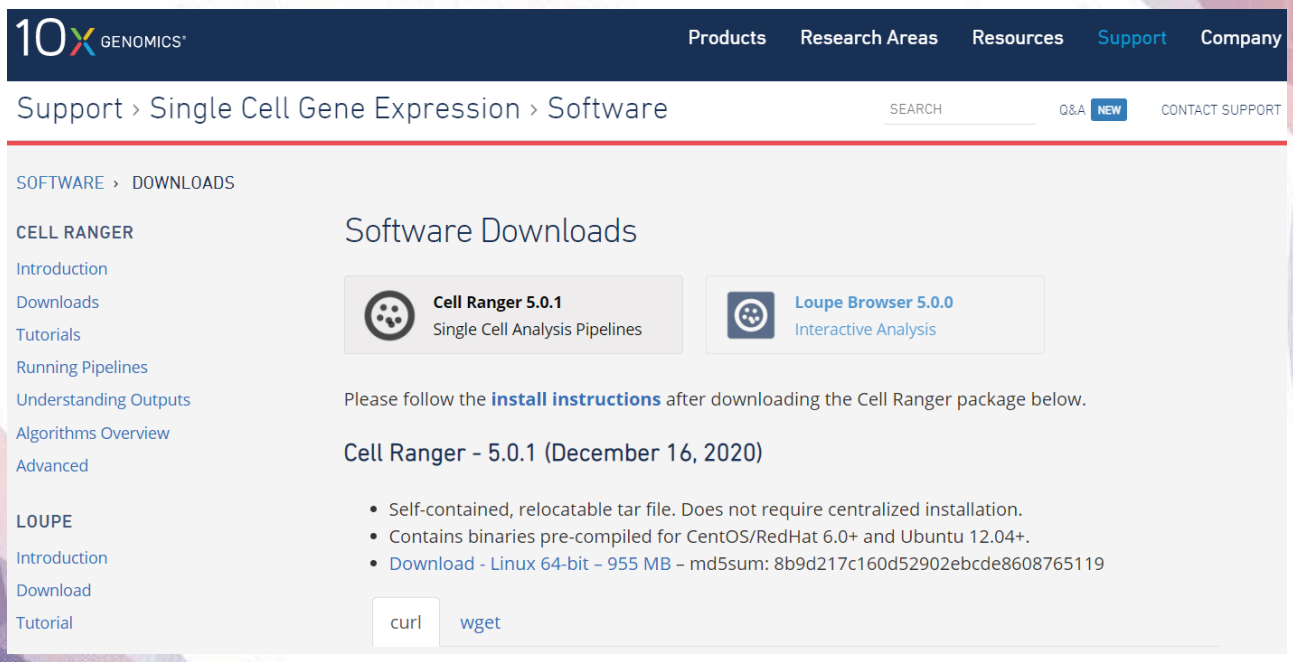

```
Library1_S1_L001_R1_001.fastq.gz
Library1_S1_L001_R2_001.fastq.gz
Library1_S1_L001_I1_001.fastq.gz
```




```
@ST-E00104:1062:H2NV3CCX2:2:1101:4980:1959 2:N:0:ACAGAGGT
NCACTTTCTGTTTTCGGATTGAAGAAGATGTACATTTTTTGTCAACTCGTACTTTTATCAGATAAGCAACTCACGTATTTGGATCTTTATT
+
#AA-A-AAAJJJJ-7JF<FFJ-7FA-AFJJJJJ--<-<AFJ-<FJ<AA--F7A7<-A<A--A--7--7A-<7---77-A<<---7AFA---
@ST-E00104:1062:H2NV3CCX2:2:1101:5446:1959 2:N:0:ACAGAGGT
NTCCCGCCCGCGCCGCCTCTTAGAGACTCGGGTCTTCTGTTCCACACGTCGGTTTCGGTGACCGATATTGTTGTCACTGCTGCGGGGTAAG
+
#AA-AFAJJ<-AJF<JF-7A--7<<7F--7A-77---<-F<-<FJ-J<-FJAFA-7A<-A-A------<-7-7------<F<7A-----7-
@ST-E00104:1062:H2NV3CCX2:2:1101:5507:1959 2:N:0:ACAGAGGT
NATGAATAAGAGGTGGACACAACAGCATGCTCCGGCAGCACGGCGTGGTGTGTCCCTGGACACATCCCTTCATTCCATGGACTTAGAGGCG
+
#-AAA7FJJJ<JFJ7FJJ7<J-FJAJJ-<FFFJ-77FAFJJJ<AAAJAA-F-FJ-FJA-FAA-A7A-77AA-A-<7F<-F7A-A<F<<FF-
```

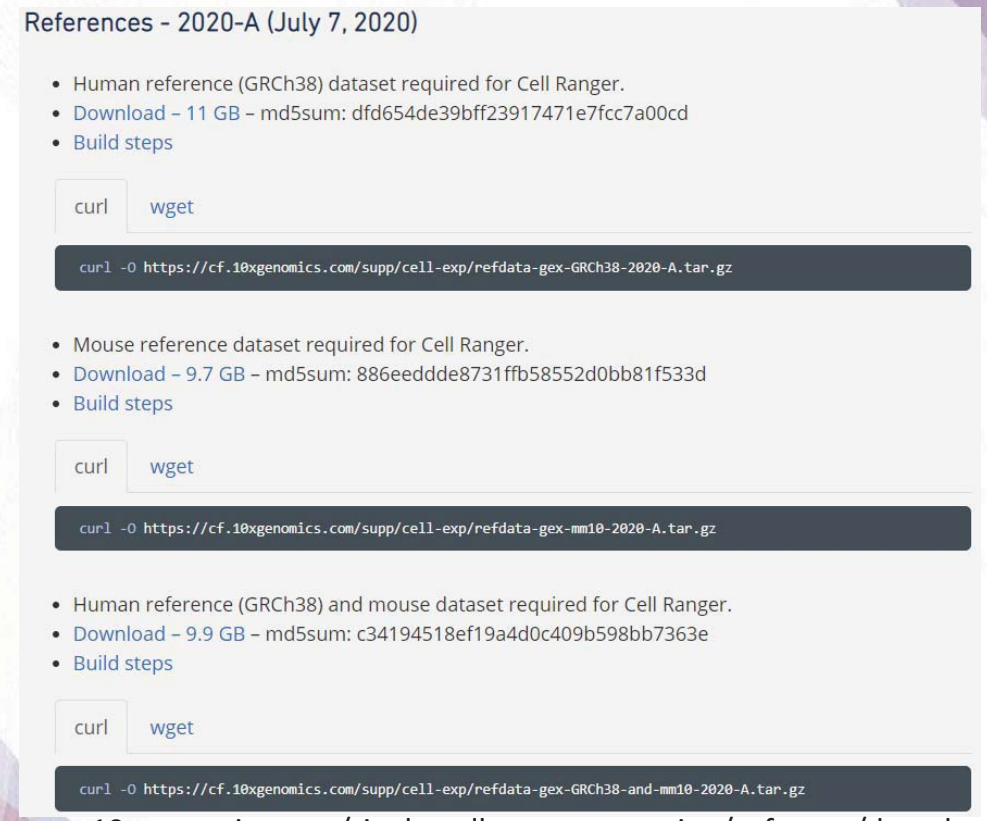
18

Cell Ranger



https://support.10xgenomics.com/single-cell-gene-expression/software/downloads/latest

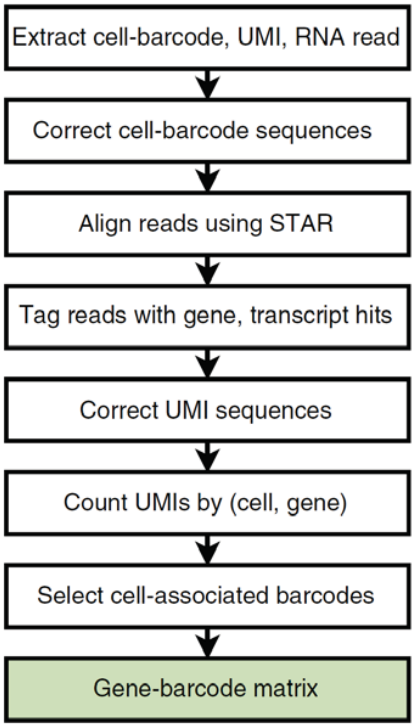
19



https://support.10xgenomics.com/single-cell-gene-expression/software/downloads/latest

20

Cell Ranger pipeline workflow



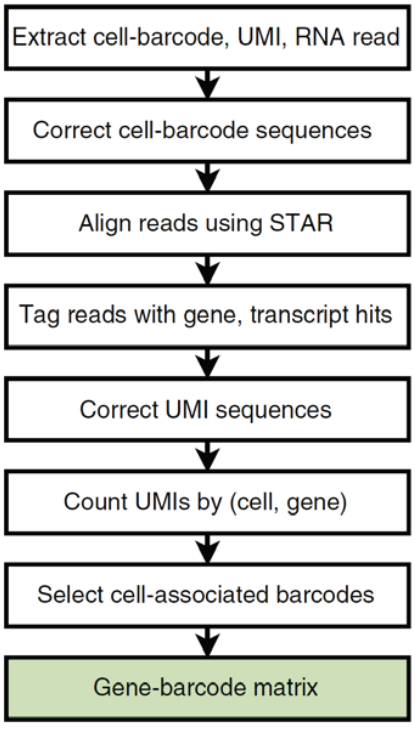

```
$ cellranger count \
--id=sample-1a \
--transcriptome=refdata-cellranger-hg19-1.2.0 \
--fastqs=sample-1a/ \
--sample=sample-1a \
--localcores 20 \
--localmem 24
```

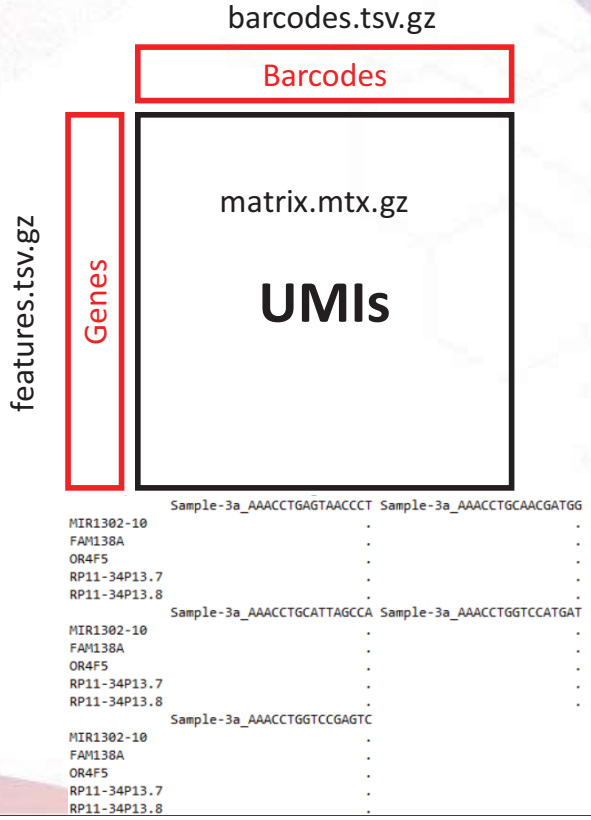
Output directory
[OUTPUTDIR] /outs/filtered_feature_bc_matrix/

1. barcodes.tsv.gz
2. features.tsv.gz
3. matrix.mtx.gz

21

Cell Ranger pipeline workflow





22

[OUTPUTDIR] /outs/web_summary.html

Estimated Number of Cells

4,143

Mean Reads per Cell	Median Genes per Cell
13,843	616

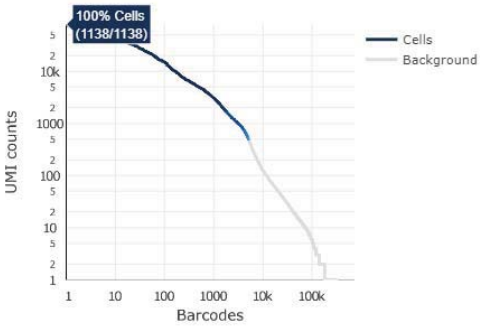
Sequencing

Number of Reads	57,353,853
Valid Barcodes	98.4%
Sequencing Saturation	34.4%
Q30 Bases in Barcode	97.4%
Q30 Bases in RNA Read	80.6%
Q30 Bases in UMI	97.1%

Mapping

Reads Mapped to Genome	64.9%
Reads Mapped Confidently to Genome	62.7%
Reads Mapped Confidently to Intergenic Regions	4.5%
Reads Mapped Confidently to Intronic Regions	11.9%
Reads Mapped Confidently to Exonic Regions	46.3%
Reads Mapped Confidently to Transcriptome	44.4%
Reads Mapped Antisense to Gene	0.6%

Cells



Estimated Number of Cells	4,143
Fraction Reads in Cells	74.6%
Mean Reads per Cell	13,843
Median Genes per Cell	616
Total Genes Detected	20,190
Median UMI Counts per Cell	1,379

Sample

Name	Sample-3a
Description	
Transcriptome	hg19
Chemistry	Single Cell 3' v2
Cell Ranger Version	3.0.2

23

RStudio

Sign in to RStudio

Username:

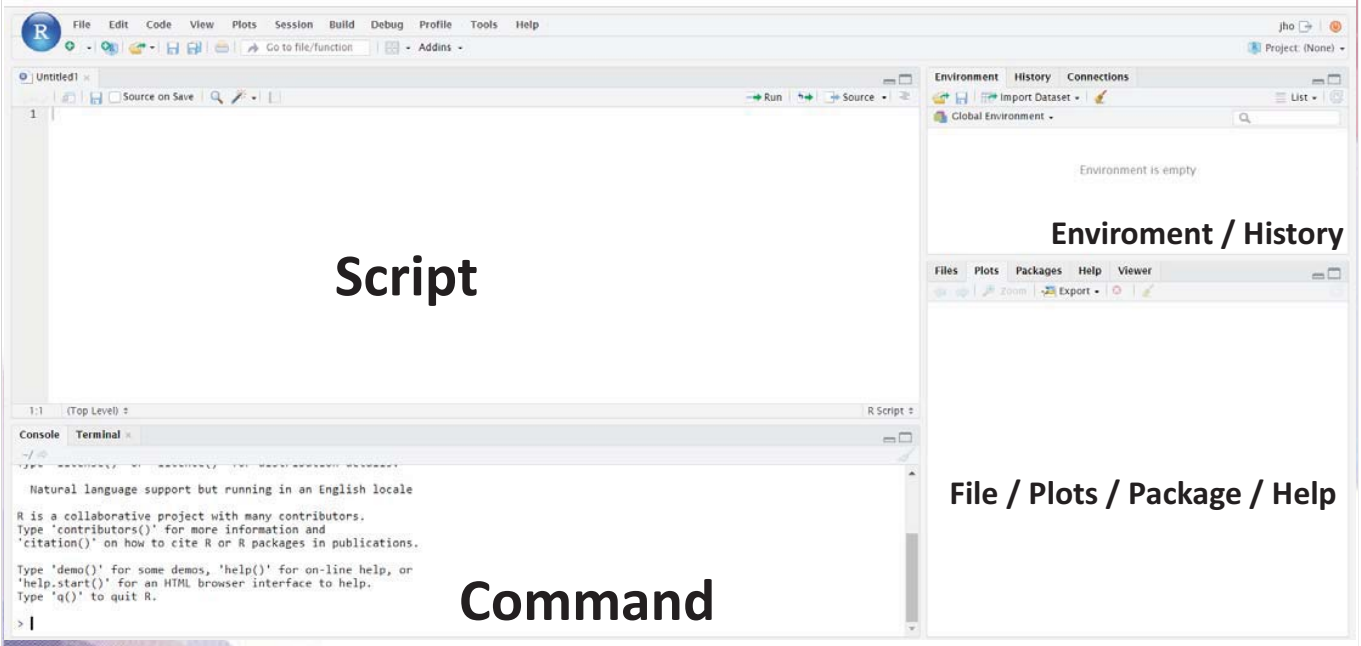
Password:

Stay signed in

Sign In

24

Rstudio



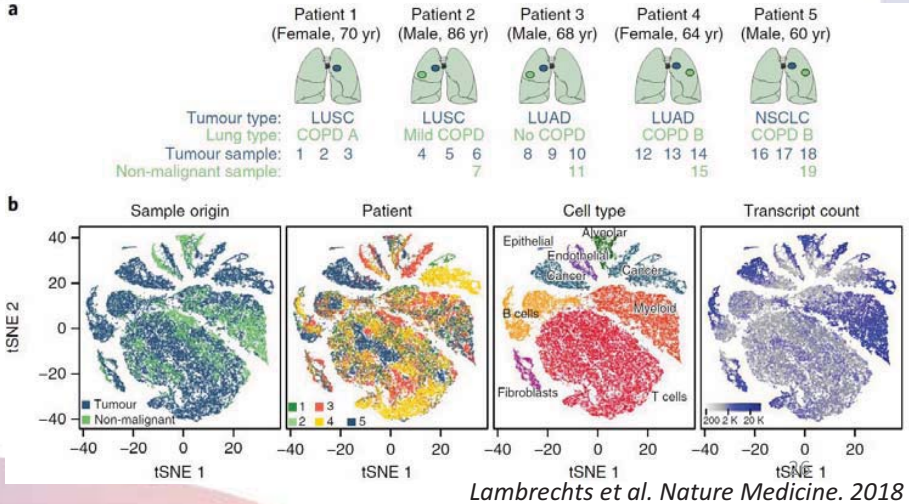
Test dataset

nature medicine
RESOURCE
https://doi.org/10.1038/s41591-018-0096-5

Phenotype molding of stromal cells in the lung tumor microenvironment

Diether Lambrechts[1,2*], Els Wauters[3,4], Bram Boeckx[1,2], Sara Aibar[5,6], David Nittner[7,8], Oliver Burton[6,9], Ayse Bassez[1,2], Herbert Decaluwé[10,11], Andreas Pircher[1,12], Kathleen Van den Eynde[13], Birgit Weynand[13], Erik Verbeken[13], Paul De Leyn[11], Adrian Liston[6,9], Johan Vansteenkiste[3,4], Peter Carmeliet[1,12,14], Stein Aerts[5,6] and Bernard Thienpont[1,15*]

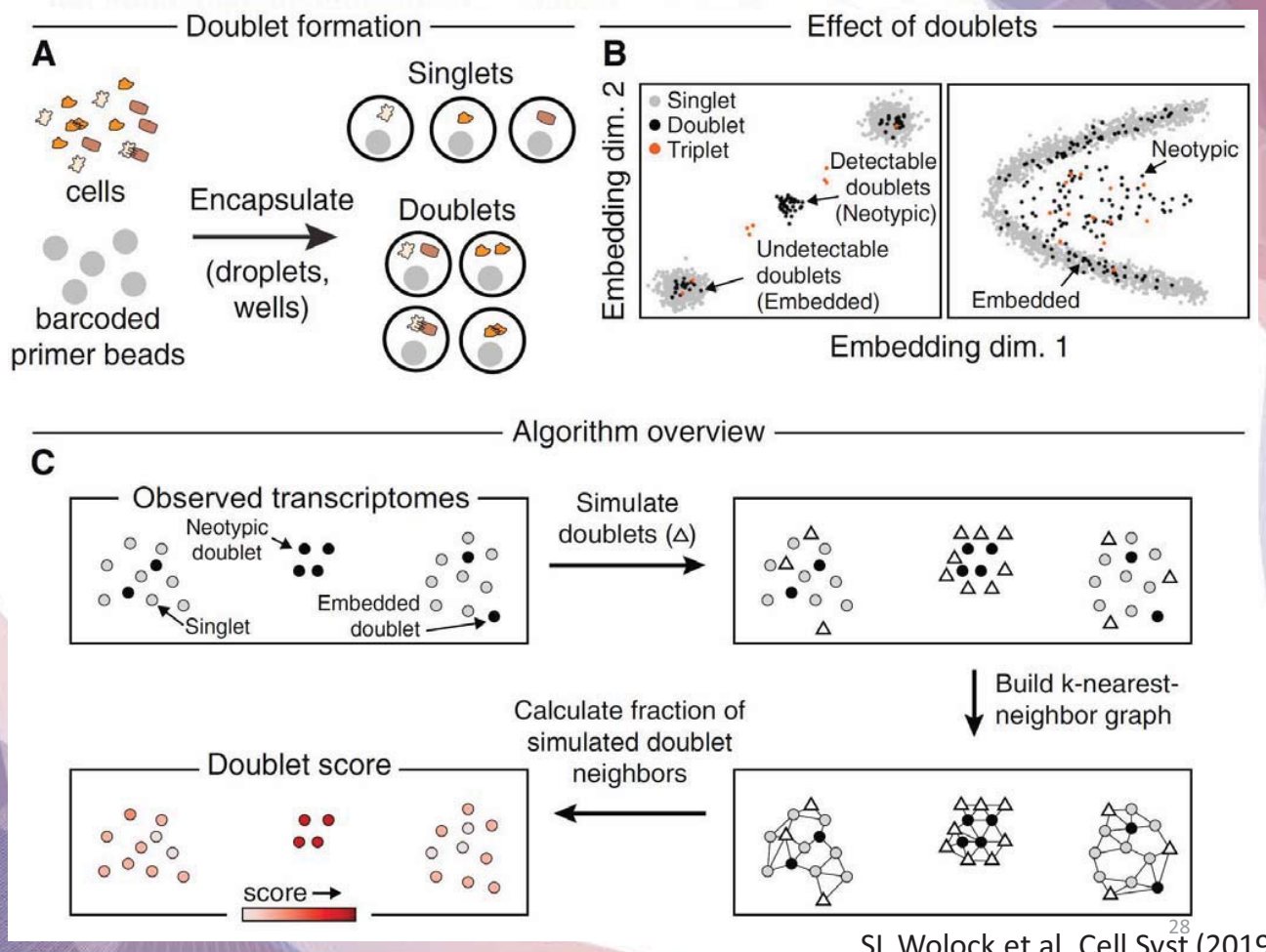
- **52,698** single cells from **lung tumors** and distal non-malignant lung samples
- Input dataset : ~/Resource/RawData/Leuven/filtered_feature_bc_matrix



Lambrechts et al. Nature Medicine. 2018

Identify doublets (Scrublet)

https://github.com/AllonKleinLab/scrublet



SL Wolock et al. Cell Syst (2019)

Load counts matrix and gene list (Python)

- import sysimport os
- import scrublet as scr
- import scipy.io
- import numpy as np
- import pandas as pd

- counts_matrix = scipy.io.mmread(input_dir + '/matrix.mtx').T.tocsc()
- genes = np.array(scr.load_genes(input_dir + '/features.tsv', delimiter = '\t', column = 1))
- barcodes = np.loadtxt(input_dir + '/barcodes.tsv', dtype = 'str')

29

Initialize Scrublet object (Python)

- scrub = scr.Scrublet(counts_matrix, expected_doublet_rate = 0.06)
  - *expected_doublet_rate*: the expected fraction of transcriptomes that are doublets, typically 0.05-0.1.
  - *sim_doublet_ratio*: the number of doublets to simulate, relative to the number of observed transcriptomes. This should be high enough that all doublet states are well-represented by simulated doublets. (default = 2)
  - *n_neighbors*: Number of neighbors used to construct the KNN classifier of observed transcriptomes and simulated doublets. (default = round(0.5*sqrt(n_cells)))

30

Run the default pipeline (Python)

- doublet_scores, predicted_doublets = scrub.scrub_doublets(min_counts = 2, min_cells = 3, min_gene_variability_pctl = 85, n_prin_comps = 30)
  - *min_gene_variability_pctl* : Keep the most highly variable genes (default: 85.0)
  - *n_prin_comps* : Number of principal components used to embed the transcriptomes prior to k-nearest-neighbor graph construction.

31

Save result (Python)

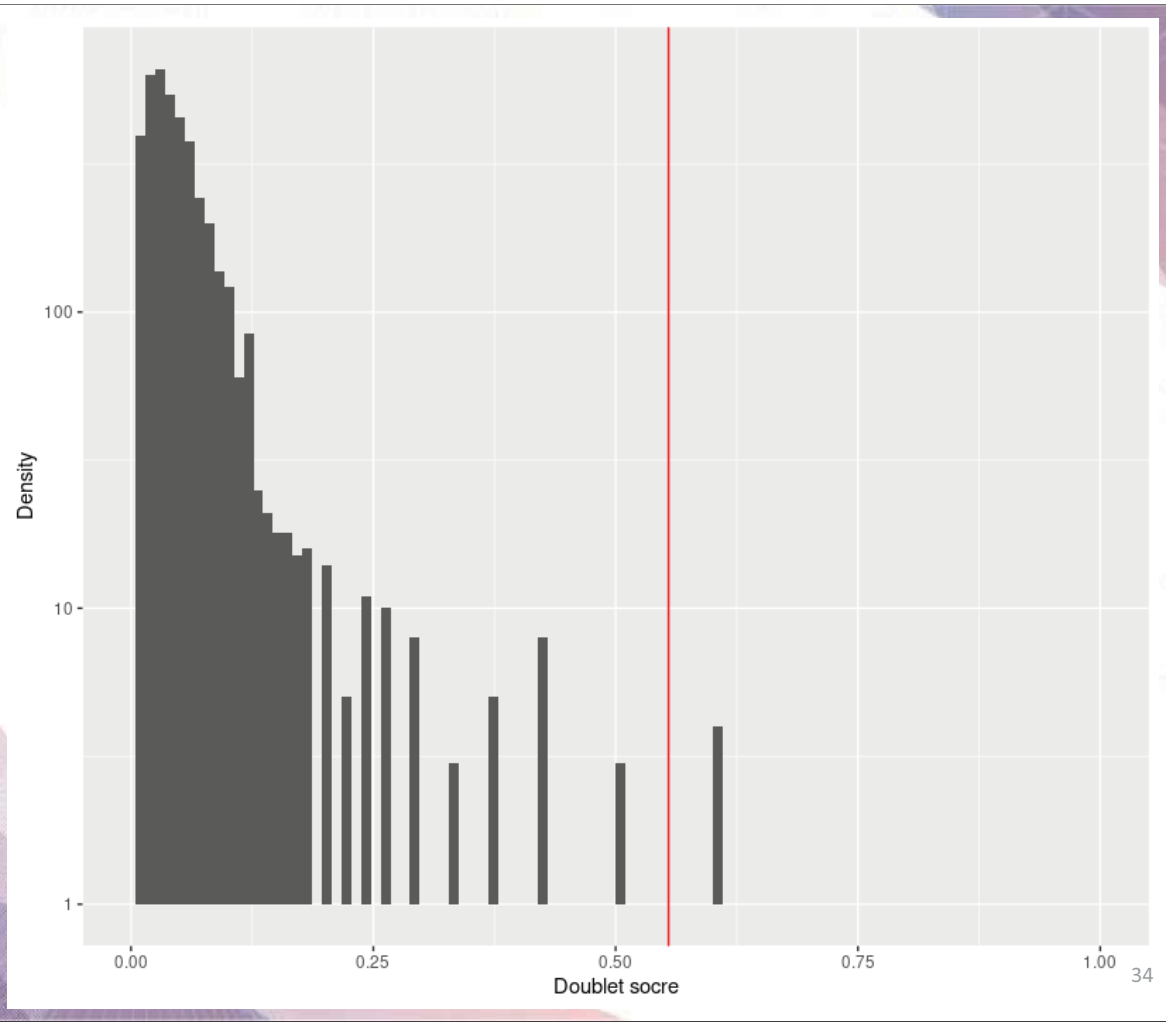
- scrub_obs = pd.DataFrame({ "barcodes" : barcodes, "doublet_scores_obs" : scrub.doublet_scores_obs_, "threshold" : scrub.threshold_})
- scrub_sim = pd.DataFrame({ "doublet_scores_sim" : scrub.doublet_scores_sim_, "threshold" : scrub.threshold_})
- scrub_obs.to_csv(out_dir + "/scrublet.doublet_scores_obs.txt", index = False, header = None, sep = "\t")
- scrub_sim.to_csv(out_dir + "/scrublet.doublet_scores_sim.txt", index = False, header = None, sep = "\t")

32

Plot doublet score histograms (R)

- db.score <- read.table(file = paste0(outdir, "/scrublet.doublet_scores_obs.txt"), header = F, sep = "\t")
- colnames(db.score) <- c("barcodes", "scores", "threshold")
- db.score$barcodes <- do.call(rbind, strsplit(as.character(db.score$barcodes), split = "\\-"))[,1]
- ggplot(data = db.score, aes(x = scores)) + geom_histogram(bins = 100) + xlim(0,1) + xlab("Doublet socre") + ylab("Density") + scale_y_continuous(trans = 'log10') + geom_vline(xintercept = unique(db.score$threshold), color = "red")

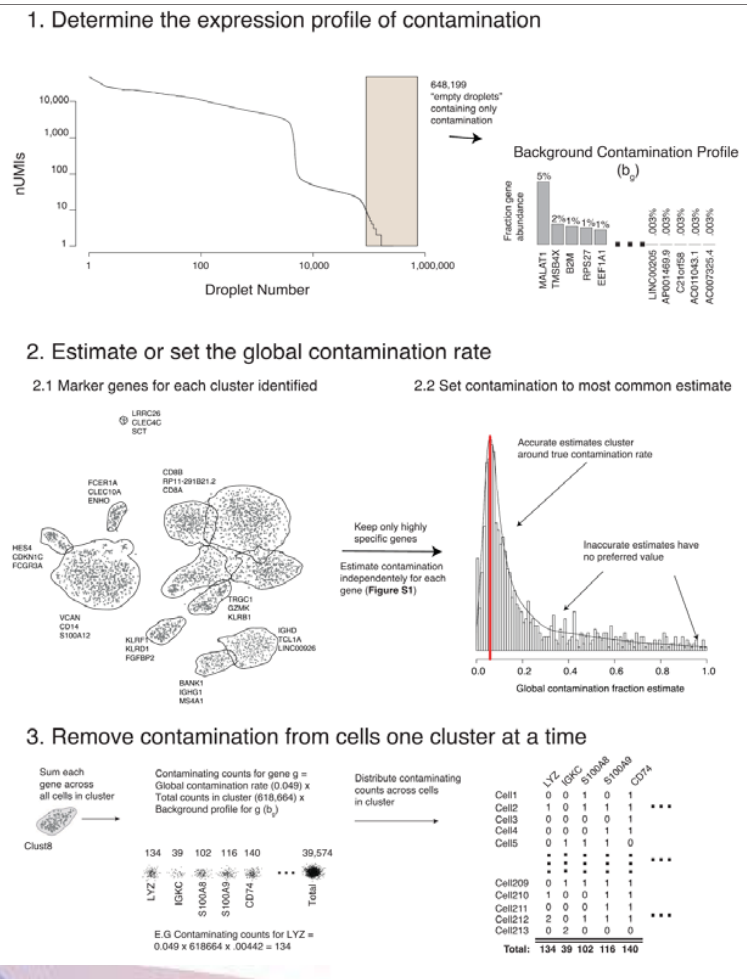
33



34

Remove cell-free mRNA contamination (SoupX)

https://github.com/constantAmateur/SoupX



MD Young et al. Gigascience (2020)

Load counts matrix and gene list

- library(SoupX)
- library(DropletUtils)
- cellranger.dir <- "~/BIML-2021-SingleCellRNAseq/Resource/RawData/Leuven“
- outdir <- "~/BIML-2021-SingleCellRNAseq/Result/SoupX“
- sc <- load10X(cellranger.dir)

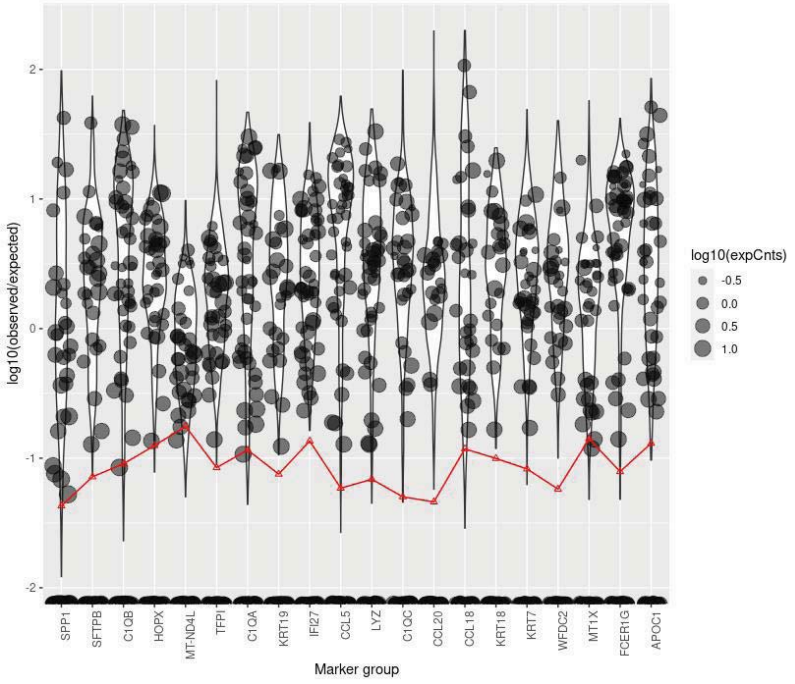
Genes to estimate the contamination fraction

- head(sc$soupProfile[order(sc$soupProfile$est, decreasing = TRUE),], n = 20)

	est	counts
MT-CO3	0.074241866	167989
MT-CO1	0.062408352	141213
MT-ND2	0.053987535	122159
MT-CO2	0.046211075	104563
MT-ND4	0.043158562	97656
MT-CYB	0.033867114	76632
MT-ATP6	0.029836578	67512
MT-ND1	0.027338706	61860
MALAT1	0.026268315	59438
MT-ND3	0.018138299	41042
TMSB4X	0.016731588	37859
FTL	0.011879476	26880
B2M	0.007407437	16761
FTH1	0.007134757	16144
PTMA	0.005107556	11557
RPL41	0.004816314	10898
RPL10	0.004653679	10530
RPS18	0.004545844	10286
RPLP1	0.004410167	9979
EEF1A1	0.004136161	9359

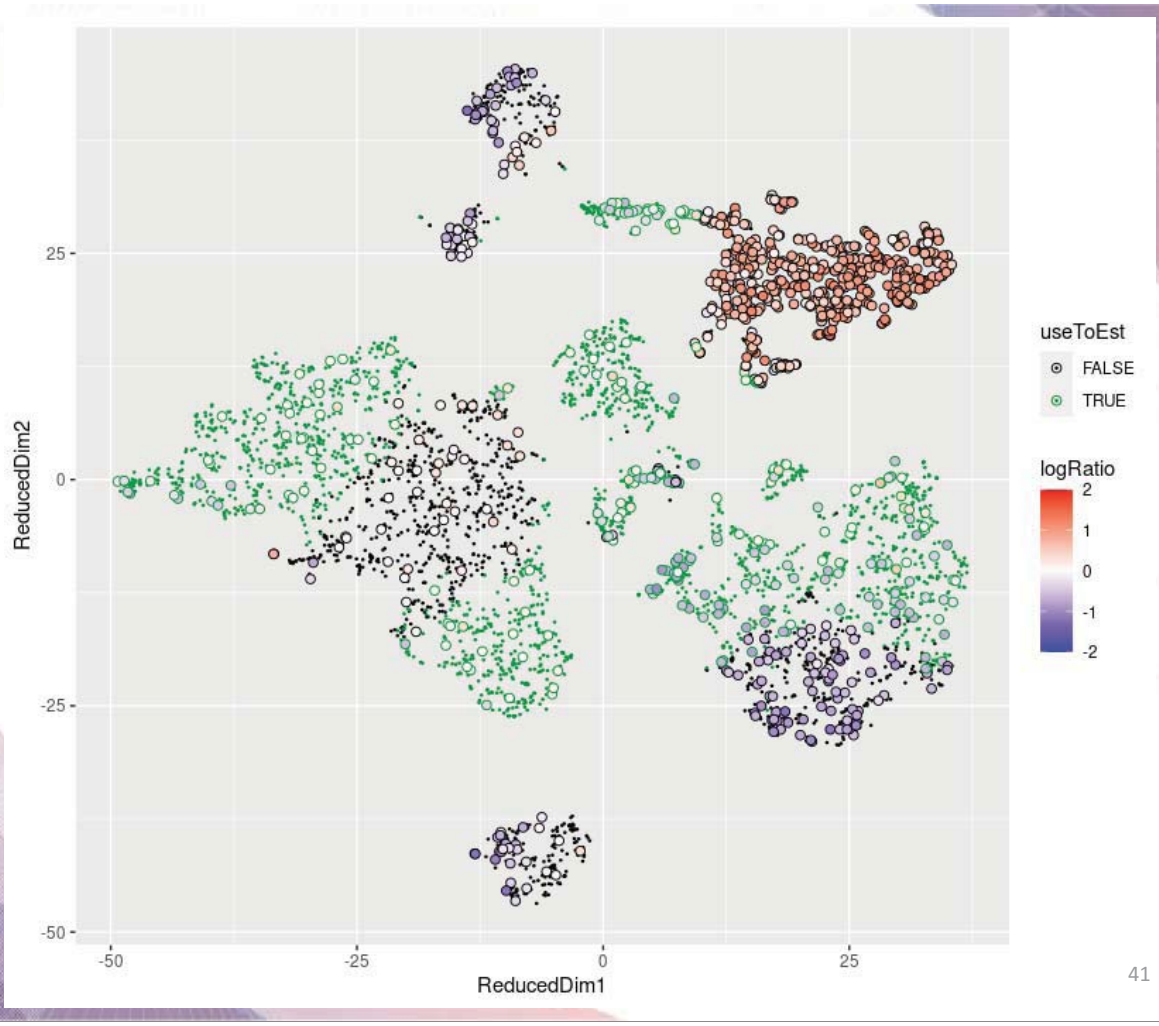
Genes to estimate the contamination fraction

- plotMarkerDistribution(sc)



Estimating non-expressing cells

- Genes <- c("KRT17", "KRT18", "KRT19")
- useToEst <- estimateNonExpressingCells(sc, nonExpressedGeneList = list(KRT = Genes))
- plotMarkerMap(sc, geneSet = Genes, useToEst = useToEst)

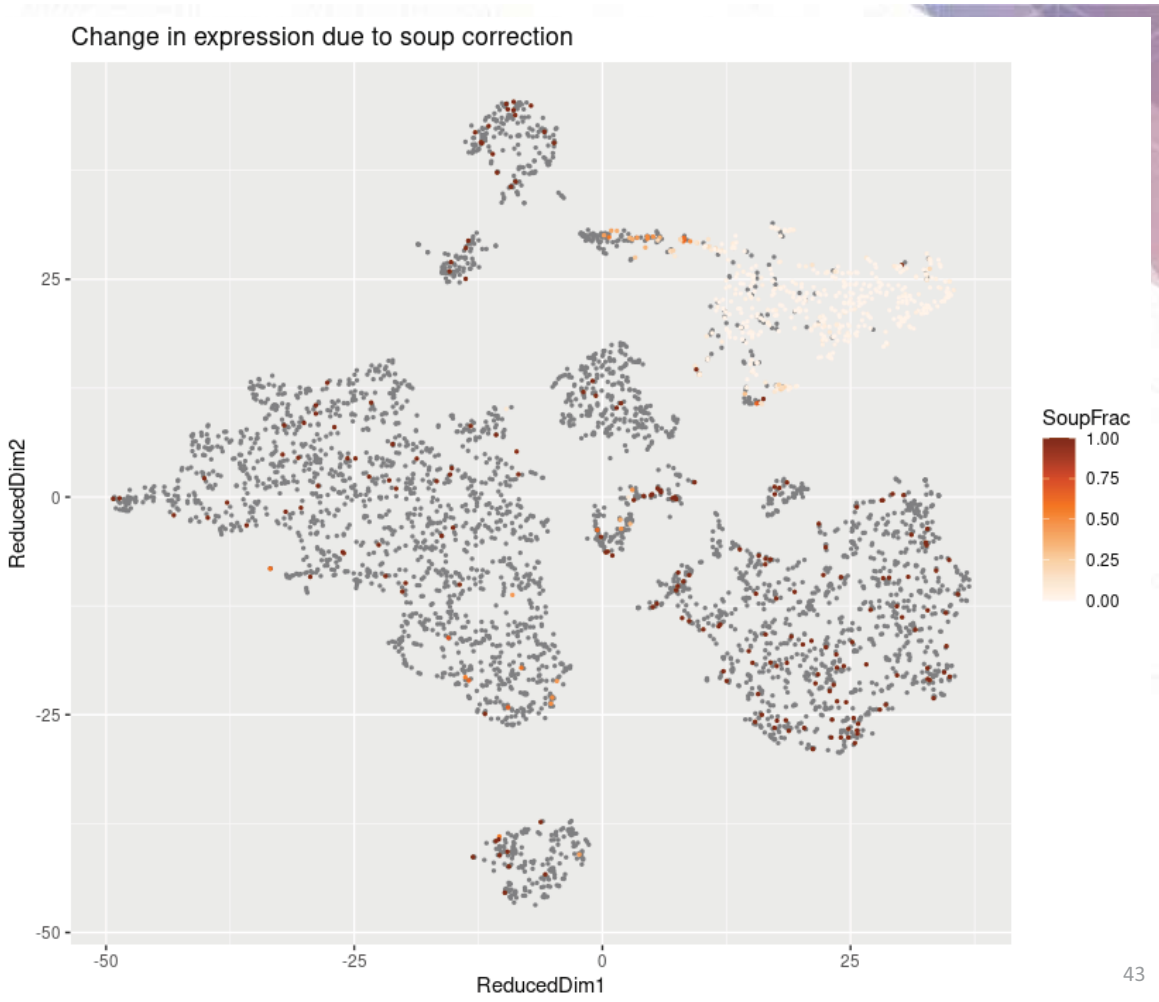


41

Calculating the contamination fraction

- sc <- calculateContaminationFraction(sc, list(KRT = Genes), useToEst = useToEst)
- out <- adjustCounts(sc)
- write10xCounts(path = paste0(outdir, "/strainedCounts"), x = out)
- plotChangeMap(sc, out, "KRT19")

42



43

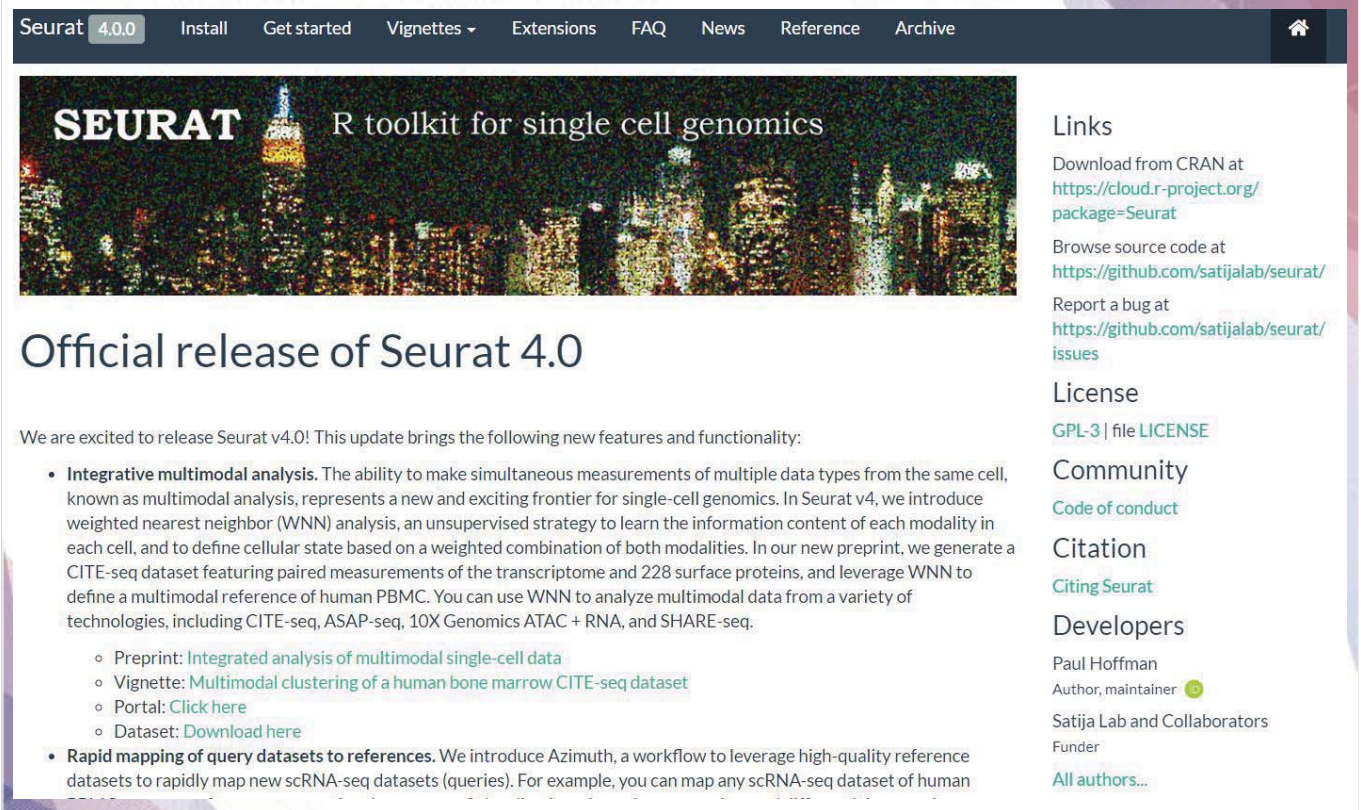
The automated method

- sc <- load10X(cellranger.dir)
- sc <- autoEstCont(sc)
- out <- adjustCounts(sc)

44

scRNA-seq analysis (Seurat)

https://satijalab.org/seurat/index.html



https://satijalab.org/seurat/

Load raw count data

- library(Seurat)
- library(plyr)
- raw.data <- Read10X(data.dir = "~/BIML-2021-SingleCellRNAseq/Result/SoupX/strainedCounts")

47

Create A Seurat Object

- seurat.obj <- CreateSeuratObject(counts = raw.data, min.cells = 3, min.features = 200)
  - **counts :** Unnormalized data such as raw counts or TPMs
  - **min.cells** : Include features detected in at least this many cells
  - **min.features** : Include cells where at least this many features are detected

48

QC metrics

- The number of unique genes detected in each cell.
  - Low-quality cells or empty **droplets** will often have **very few genes**
  - Cell **doublets** or multiplets may exhibit an **aberrantly high gene count**
- Similarly, the total number of molecules detected within a cell (correlates strongly with unique genes)
- The percentage of reads that map to the mitochondrial genome
  - **Low-quality / dying cells** often exhibit **extensive mitochondrial contamination**

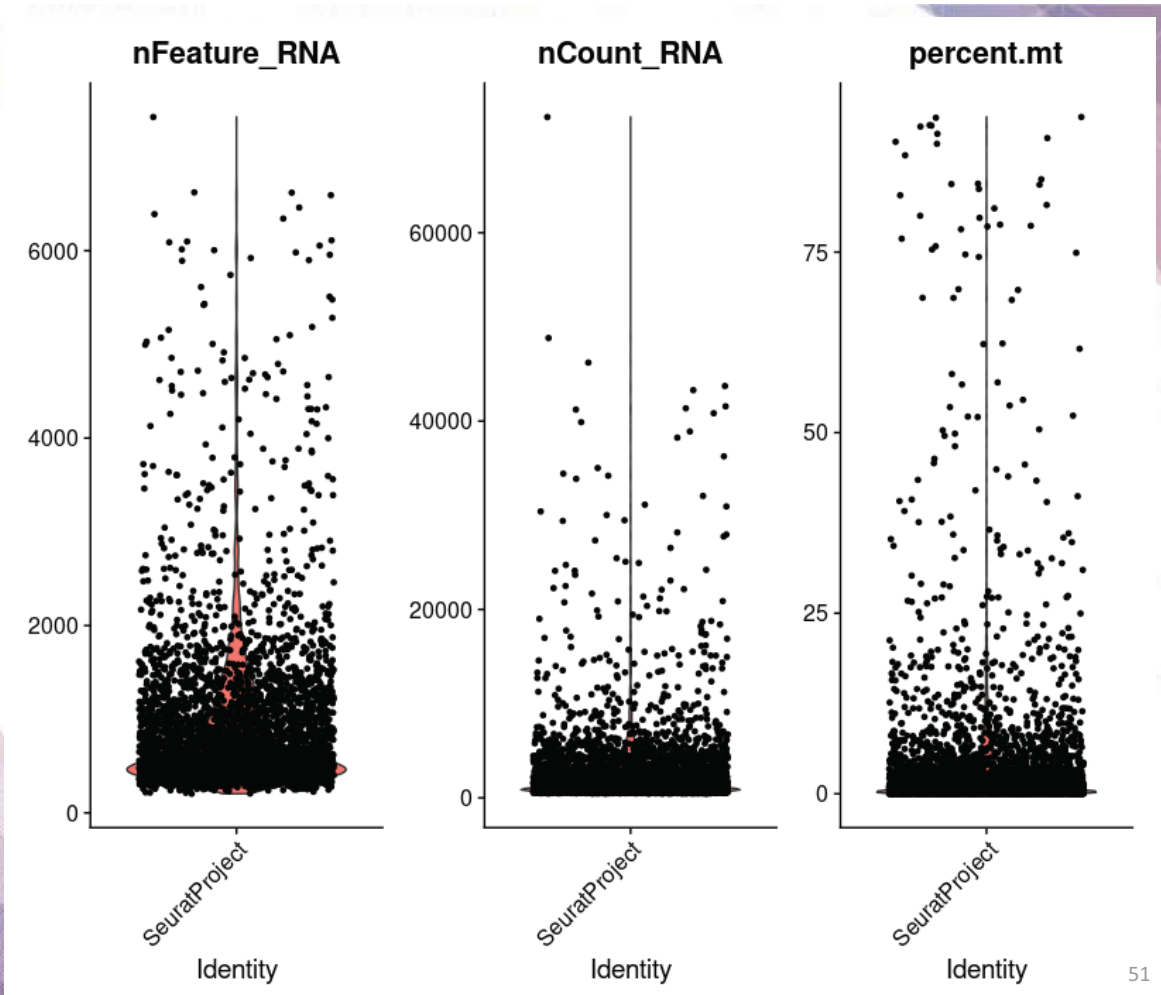
49

QC metrics

➢ seurat.obj[["percent.mt"]] <-
PercentageFeatureSet(seurat.obj, pattern = "^MT-")

➢>VlnPlot(object = seurat.obj, features =
c("nFeature_RNA", "nCount_RNA", "percent.mt"),
ncol = 3)

50

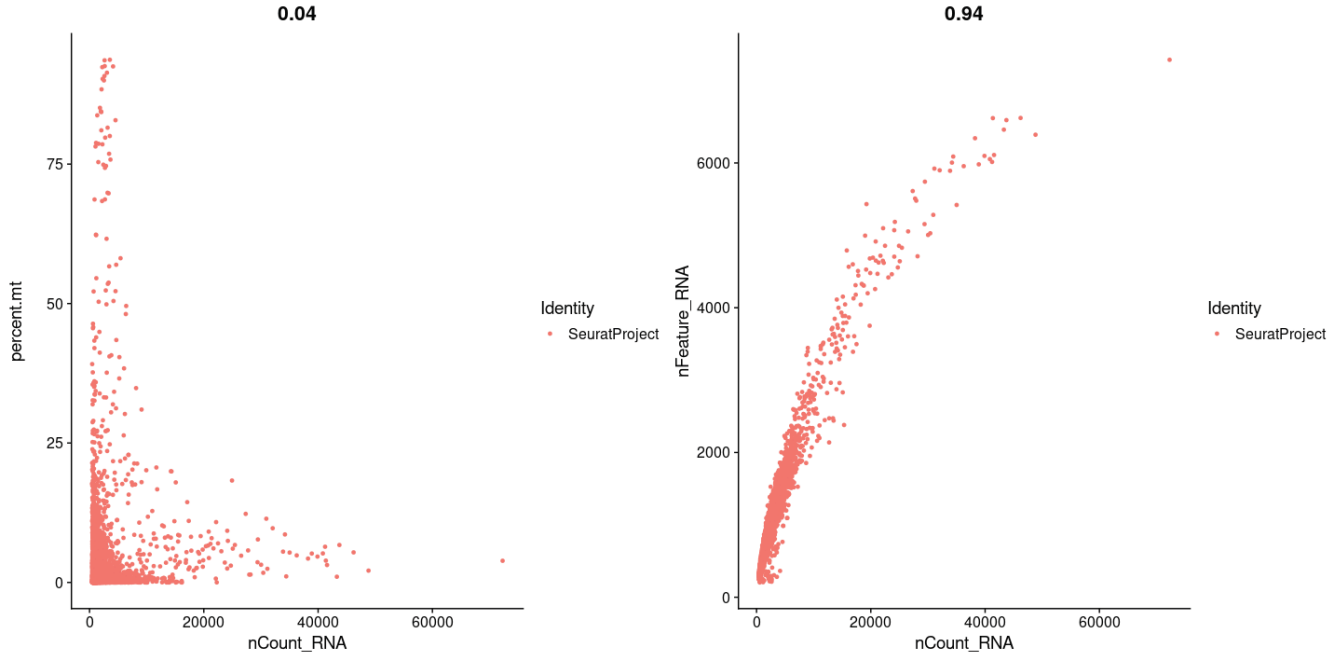


51

QC metrics

- seurat.feature.plot1 <- FeatureScatter(object = seurat.obj, feature1 = "nCount_RNA", feature2 = "percent.mt")
- seurat.feature.plot2 <- FeatureScatter(object = seurat.obj, feature1 = "nCount_RNA", feature2 = "nFeature_RNA")
- CombinePlots(plots = list(seurat.feature.plot1, seurat.feature.plot2))

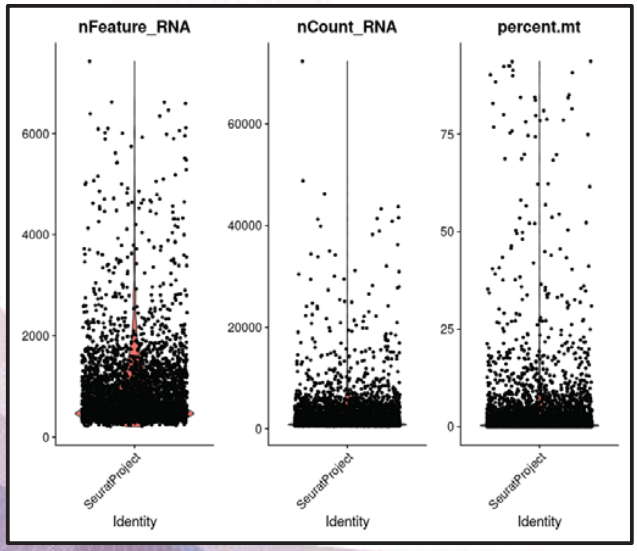
52



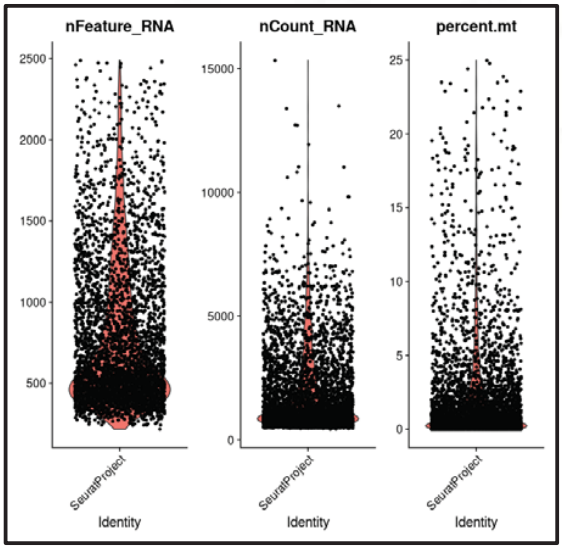
53

Filter low-quality cells

➢seurat.obj <- subset(x = seurat.obj, subset = nFeature_RNA > 200 & nFeature_RNA < 2500 & percent.mt < 25)



Before



After

54

Filtered doublet

- doublet.file <- "~/BIML-2021-SingleCellRNAseq/Result/Scrublet/scrublet.doublet_scores_obs.txt“
- doublet.df <- read.table(doublet.file , header = F, sep = "\t")
- colnames(doublet.df) <- c("barcodes", "scores", "threshold")
- doublet.df$Doublet <- "singlet“
- doublet.df$Doublet[doublet.df$scores > doublet.df$threshold] <- "doublet“

Filtered doublet

- doublet.df <- join(data.frame(barcodes = rownames(seurat.obj@meta.data), seurat.obj@meta.data), doublet.df, by = "barcodes")
- seurat.obj@meta.data$Doublet <- doublet.df$Doublet
- seurat.obj <- subset(x = seurat.obj, subset = Doublet == "singlet")

Normalizing the data

- seurat.obj <- NormalizeData(object = seurat.obj, normalization.method = "LogNormalize")
  - ***normalization.method*** : Method for normalization.
    - **LogNormalize**: Feature counts for each cell are divided by the total counts for that cell and multiplied by the scale.factor. This is then natural-log transformed using log1p.
    - **CLR**: Applies a centered log ratio transformation
    - **RC**: Relative counts. Feature counts for each cell are divided by the total counts for that cell and multiplied by the scale.factor. No log-transformation is applied. For counts per million (CPM) set scale.factor = 1e6
  - ***scale.factor*** **:** Sets the scale factor for cell-level normalization (default : 10000)

57

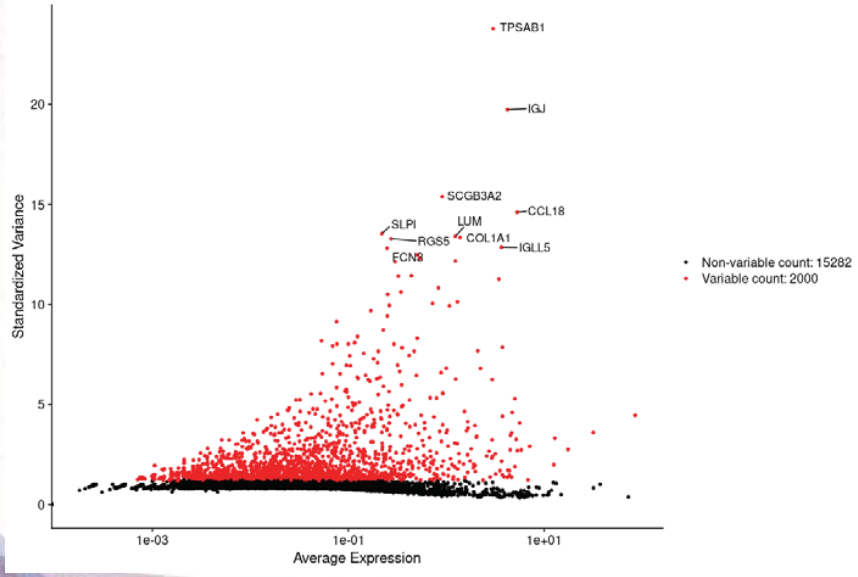
Identification of highly variable features

- seurat.obj <- FindVariableFeatures(object = seurat.obj, selection.method = "vst", nfeatures = 2000)
  - ***selection.method*** : How to choose top variable features
    - **vst** : Fits a line to the relationship of log(variance) and log(mean) using local polynomial regression (loess). Feature variance is then calculated on the standardized values after clipping to a maximum
    - **mean.var.plot (mvp)** : Divides features into num.bin (deafult 20) bins based on their average expression, and calculates z-scores for dispersion within each bin
    - **dispersion (disp)** : selects the genes with the highest dispersion values
  - ***nfeatures*** : Number of features to select as top variable features; only used when selection.method is set to *'dispersion'* or *'vst'*

58

Identification of highly variable features

- v.genes <- VariableFeatures(object = seurat.obj)
- LabelPoints(plot = VariableFeaturePlot(seurat.obj), points = v.genes[1:10], repel = T)



Scaling the data

- seurat.obj <- ScaleData(object = seurat.obj, features = rownames(seurat.obj), vars.to.regress = "percent.mt")
  - ***features*** : Vector of features names to scale/center (Default : all features)
  - ***vars.to.regress*** : Variables to regress out. For example, nUMI, or percent.mito. (Optional)
  - ***model.use*** : Use a linear model or generalized linear model for the regression Options are 'linear', 'poisson', and 'negbinom'(default : linear)

Dimensional reduction

➢seurat.obj <- RunPCA(object = seurat.obj, features = v.genes)

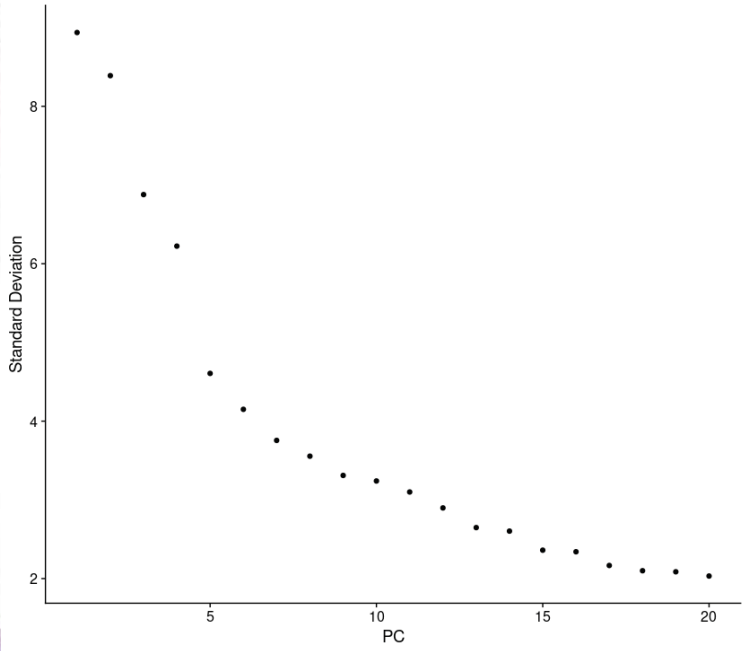
- ***npcs*** : Total Number of PCs to compute and store (default : 50)
- ***features*** : Features to compute PCA on

```
PC_ 1
Positive:  TYROBP, CD74, HLA-DPB1, FCER1G, HLA-DRA, AIF1, ALOX5AP, HLA-DPA1, LYZ, SRGN
           C1orf162, HLA-DRB1, HLA-DQA1, CD68, C1QA, C1QB, HLA-DQB1, MS4A6A, MS4A4A, CTSS
           CAPG, MS4A7, C1QC, FCGR3A, RNASE6, LST1, HLA-DRB5, CD14, APOC1, HLA-DMB
Negative:  SPARC, DCN, CALD1, COL1A2, BGN, C1R, COL6A2, SPARCL1, COL3A1, RARRES2
           C1S, MGP, IGFBP7, LUM, COL1A1, A2M, NNMT, COL6A3, PCOLCE, MYL9
           THY1, COL6A1, TPM2, MMP2, IGFBP4, COL5A2, MFAP4, SFRP2, AEBP1, TAGLN
PC_ 2
Positive:  CCL5, IL32, CD7, GZMA, GZMB, NKG7, PRF1, CTSW, CCL4, KLRB1
           CD69, CD27, HOPX, TIGIT, GNLY, CD8A, ITM2A, IFNG, CXCL13, GZMH
           KLRD1, TNFRSF18, LTB, GZMK, CD8B, BIRC3, KRT19, KRT18, SFTPB, KRT7
Negative:  CST3, CD68, LYZ, AIF1, FCER1G, TYROBP, CTSB, FTL, CTSL, MS4A4A
           GPNMB, LGALS1, C1QA, C1QB, TIMP1, PSAP, GLUL, GPX1, MS4A7, GRN
           APOC1, FTH1, FCGRT, C1QC, CD14, MS4A6A, APOE, C1orf162, TMEM176B, CTSS
PC_ 3
Positive:  KRT19, KRT18, KRT7, WFDC2, SFTPB, AGR2, SLC34A2, CCND1, KRT8, EPCAM
           SPINT2, MALL, SFTA2, TACSTD2, UBE2C, S100A14, HOPX, MGST1, CLDN4, HMGB3
           ERRFI1, NAPSA, PAEP, GPRC5A, TFPI, AQP3, SDR16C5, SPINK1, LSR, NPC2
Negative:  CCL5, IL32, COL1A2, SRGN, DCN, COL3A1, GZMA, COL6A2, CD7, LUM
           NKG7, GZMB, RARRES2, SFRP2, COL5A2, CCL4, COL6A3, PCOLCE, CTSW, BGN
           C1S, CD69, RGS1, CALD1, MFAP4, COL14A1, PRF1, AEBP1, TPM2, IGF1
PC_ 4
Positive:  RAMP2, CLEC14A, CLDN5, PLVAP, VWF, ECSCR, RAMP3, PCDH17, ELTD1, EGFL7
           CALCRL, DARC, HSPG2, EMCN, FCN3, TSPAN7, AQP1, ESAM, GNG11, HYAL2
           CCL14, CYYR1, CD34, CD93, JAM2, FAM167B, ARHGAP29, LDB2, CDH5, SPARCL1
Negative:  COL1A1, LUM, DCN, RARRES2, COL3A1, COL1A2, SFRP2, COL6A3, SERPINF1, C1S
           MFAP4, FBLN1, IGF1, PCOLCE, CCDC80, COL14A1, NBL1, COL5A2, FGF7, COL5A1
           MOXD1, COL8A1, MXRA8, RARRES1, MEG3, COL6A1, ISLR, AEBP1, FMOD, OLFML3
PC_ 5
Positive:  MMP2, SFRP2, IGF1, PTGDS, LUM, SERPINF1, MOXD1, POSTN, FGF7, FBLN1
           C7, CCDC80, COL8A1, LXN, RARRES1, LSAMP, COL1A1, ISLR, FBLN2, CTHRC1
           PLA2G2A, DPYSL3, MFAP4, COL16A1, VCAN, FFEMP1, CXCL12, NBL1, C3, IGFBP4
Negative:  HIGD1B, NDUFA4L2, COX4I2, RGS5, PTN, PPP1R14A, GJA4, PDGFRB, EGFL6, LHFP
           CSRP2, KCNJ8, NOTCH3, FOXS1, TPPP3, SEPT4, FAM162B, MAP1B, NR2F2, SMOC2
           KCNK3, GJC1, MYO1B, PTP4A3, CCDC102B, KCNK17, FRZB, TINAGL1, TBX2, LAMC3
```

61

Determine the 'dimensionality'

➢ElbowPlot(object = seurat.obj)



62

Determine the ‘dimensionality’

➢seurat.obj <- JackStraw(object = seurat.obj, num.replicate = 100)

- ***dims*** : Number of PCs to compute significance for
- ***num.replicate*** : Number of replicate samplings to perform

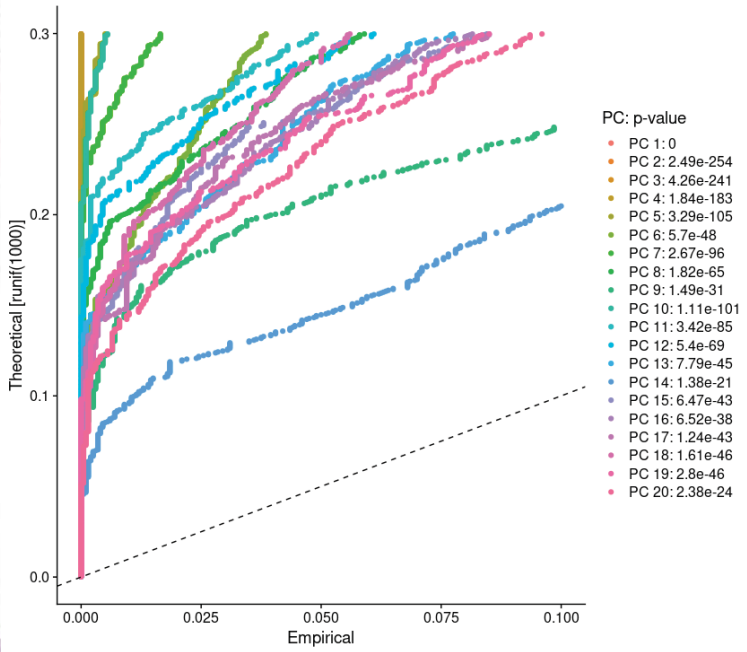
➢JackStrawPlot(object = seurat.obj, dims = 1:20)

- ***dims*** : Which dimensions to examine

63

Determine the ‘dimensionality’

➢JackStrawPlot(object = seurat.obj, dims = 1:20)



64

Clustering analysis

- seurat.obj <- FindNeighbors(object = seurat.obj, dims = 1:17)
- seurat.obj <- FindClusters(object = seurat.obj, resolution = 0.5)

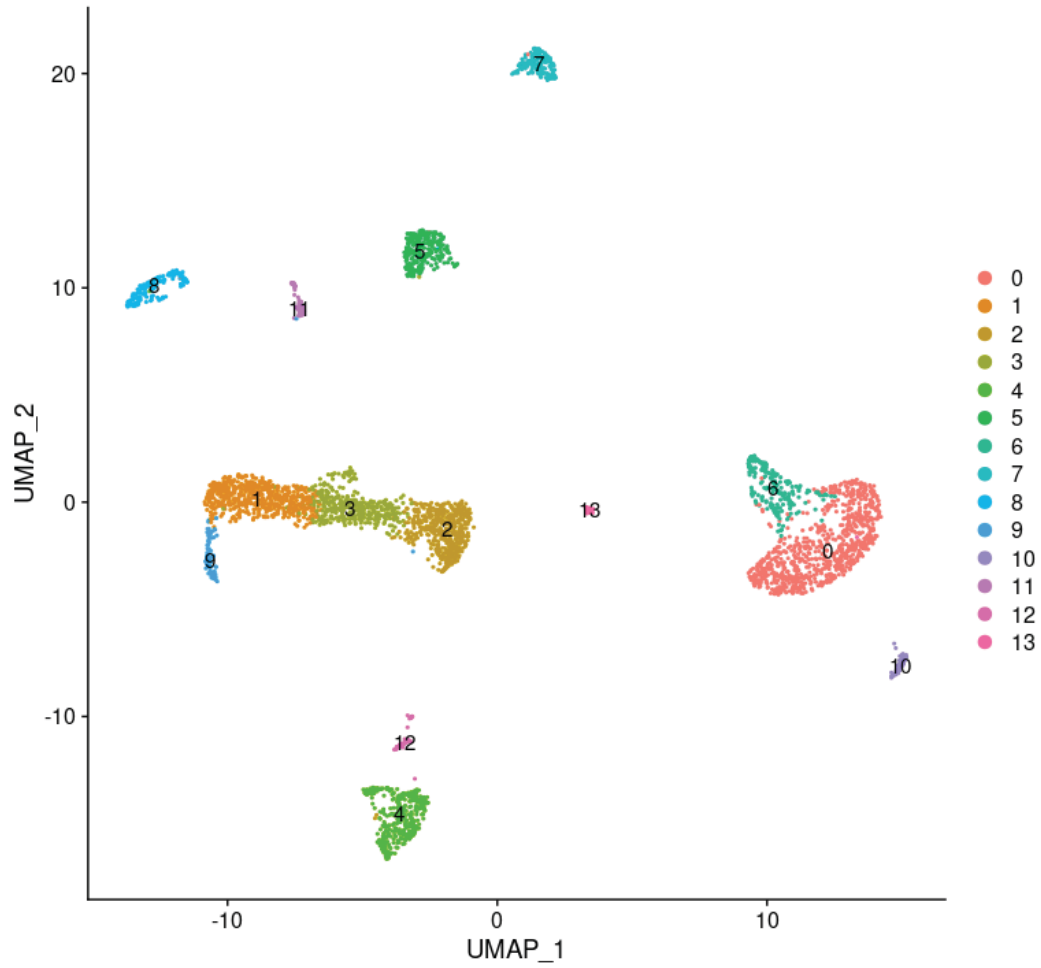
65

Run non-linear dimensional reduction

Option 1. Run UMAP

- seurat.obj <- RunUMAP(object = seurat.obj, dims = 1:17)
- DimPlot(object = seurat.obj, reduction = "umap")

66



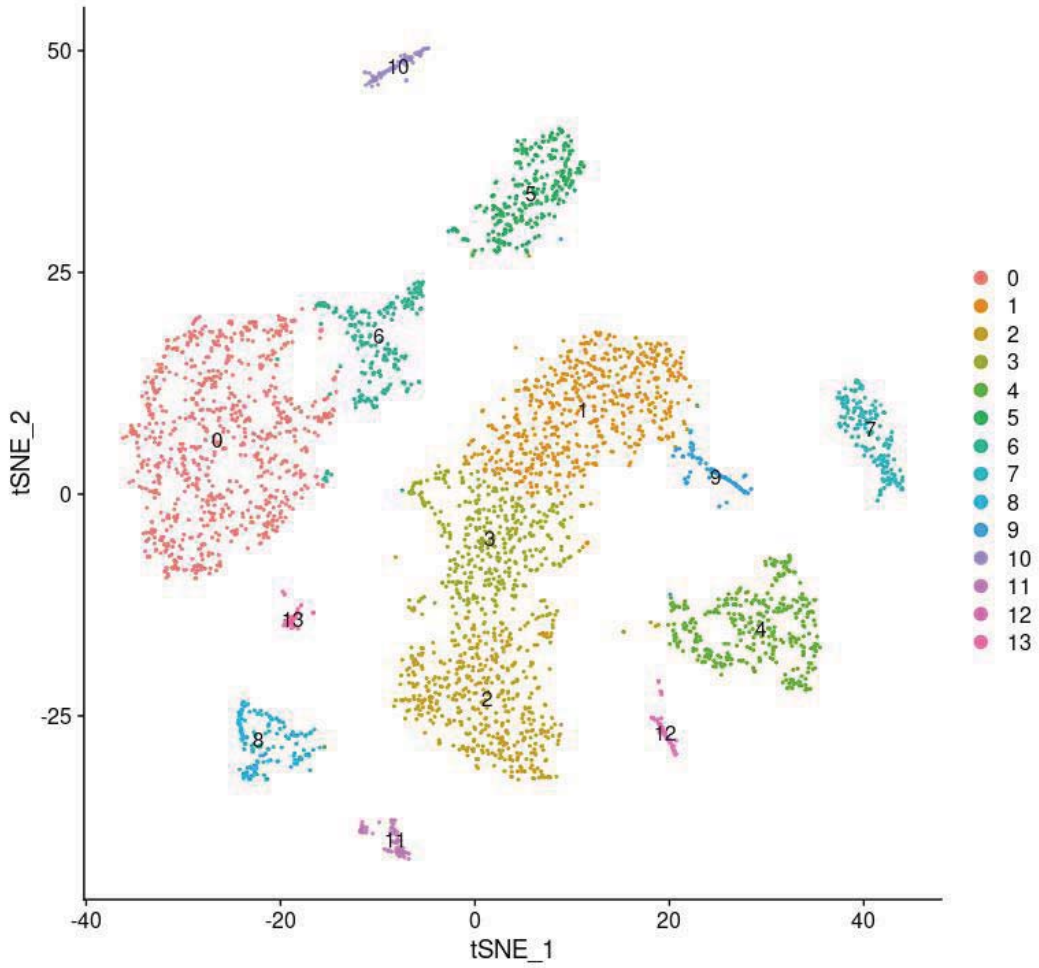
67

Run non-linear dimensional reduction

Option 2. T-Distributed Stochastic Neighbor Embedding (tSNE)

- seurat.obj <- RunTSNE(object = seurat.obj, dims = 1:17)
- DimPlot(object = seurat.obj, reduction = "tsne")

68



Save Seurat object

- saveRDS(object = seurat.obj, file = "~/BIML-2021-SingleCellRNAseq/Result/Seurat/Seurat.RDS")

Finding differentially expressed features

➢ seurat.markers <- FindAllMarkers(object = seurat.obj, only.pos = T, min.pct = 0.25, logfc.threshold = 0.25)

- ***logfc.threshold*** : Limit testing to genes which show, on average, at least X-fold difference (log-scale) between the two groups of cells (Default : 0.25)
- ***test.use*** : Denotes which test to use.
  - *wilcox* (default), *bimod*, *roc*, *t*, *negbinom*, *poisson*, *LR*, *MAST*, *DESeq2*
- ***min.pct*** : only test genes that are detected in a minimum fraction of min.pct cells in either of the two populations (Default : 0.1)
- ***only.pos*** : Only return positive markers (default : FALSE)

71

Finding differentially expressed features

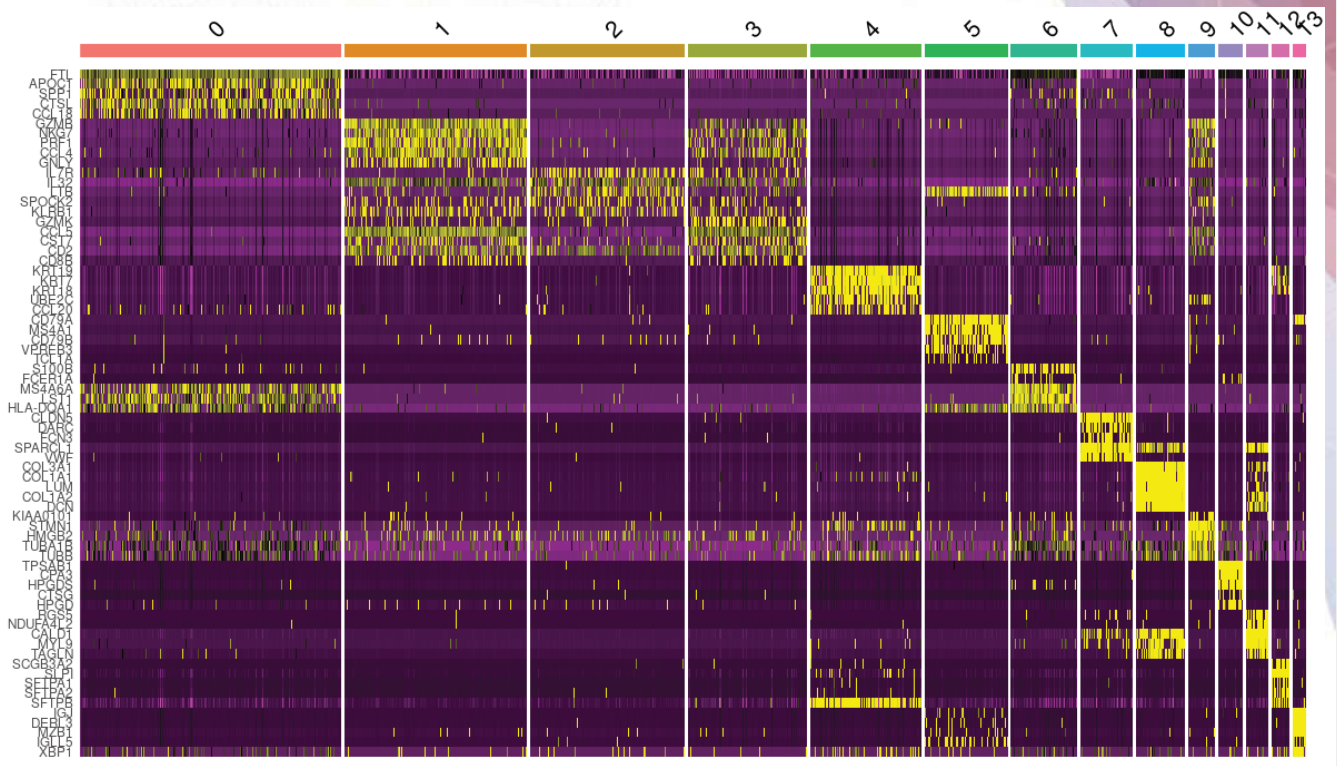
	p_val	avg_logFC	pct.1	pct.2	p_val_adj	cluster	gene
FTL	0.000000e+00	3.2485606	1.000	0.792	0.000000e+00	0	FTL
APOC1	0.000000e+00	3.1473435	0.787	0.086	0.000000e+00	0	APOC1
SPP1	0.000000e+00	3.0291203	0.577	0.027	0.000000e+00	0	SPP1
CTSL	0.000000e+00	2.5713692	0.803	0.089	0.000000e+00	0	CTSL
LYZ	0.000000e+00	2.5071625	0.933	0.061	0.000000e+00	0	LYZ
FCER1G	0.000000e+00	2.4770294	0.986	0.145	0.000000e+00	0	FCER1G
CD68	0.000000e+00	2.4704571	0.888	0.088	0.000000e+00	0	CD68
APOE	0.000000e+00	2.4695281	0.626	0.062	0.000000e+00	0	APOE
CTSB	0.000000e+00	2.4049268	0.925	0.224	0.000000e+00	0	CTSB
C1QB	0.000000e+00	2.3862444	0.730	0.081	0.000000e+00	0	C1QB
C1QA	0.000000e+00	2.3593286	0.751	0.097	0.000000e+00	0	C1QA
S100A8	0.000000e+00	2.3561258	0.628	0.028	0.000000e+00	0	S100A8
CTSD	0.000000e+00	2.3253321	0.941	0.419	0.000000e+00	0	CTSD
TYROBP	0.000000e+00	2.3151500	0.994	0.174	0.000000e+00	0	TYROBP
GLUL	0.000000e+00	2.2266139	0.917	0.183	0.000000e+00	0	GLUL
FTH1	0.000000e+00	2.1965144	1.000	0.901	0.000000e+00	0	FTH1
GPNMB	0.000000e+00	2.1177286	0.756	0.040	0.000000e+00	0	GPNMB
AIF1	0.000000e+00	2.0594121	0.937	0.076	0.000000e+00	0	AIF1

72

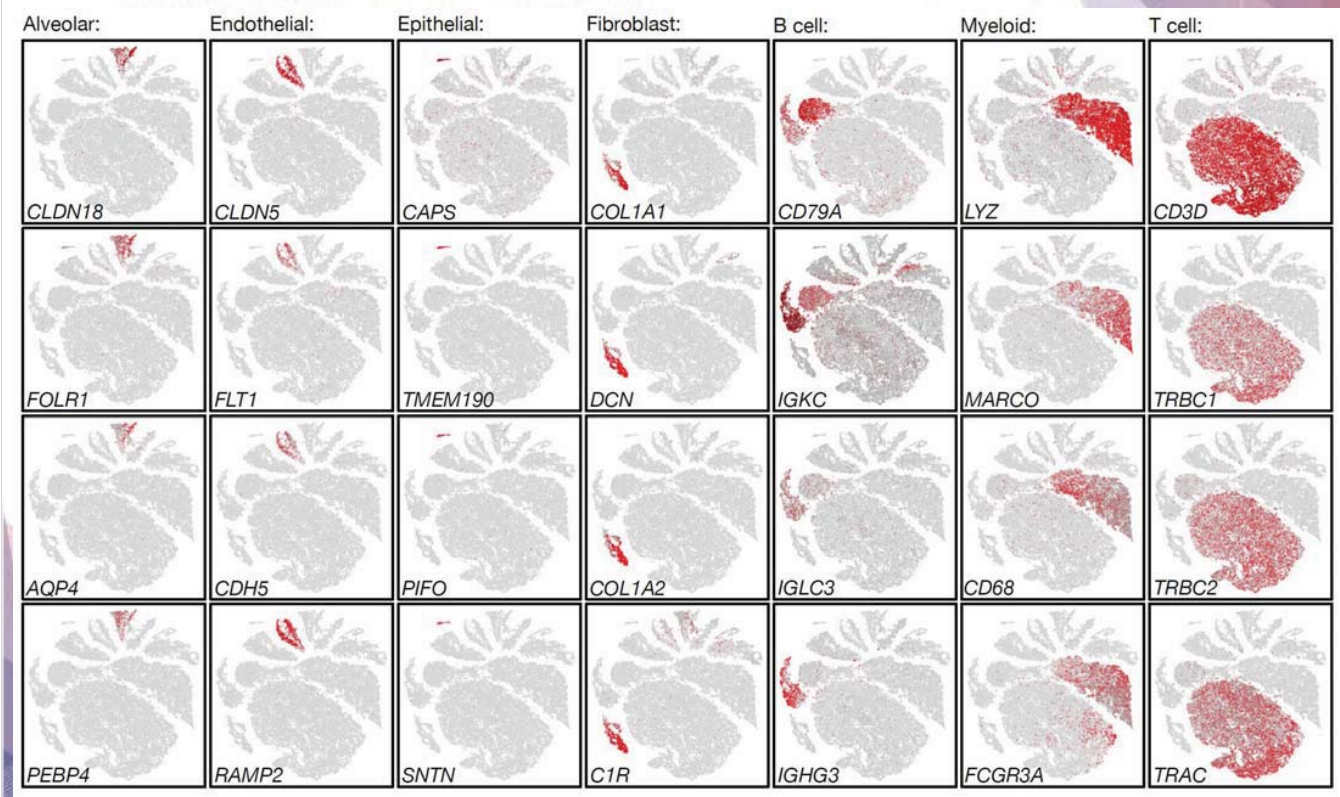
Finding differentially expressed features

- seurat.top.genes <- seurat.markers %>% group_by(cluster) %>% top_n(n = 10, wt = avg_logFC)
- DoHeatmap(object = seurat.obj, features = seurat.top.genes$gene) + NoLegend()

73



74

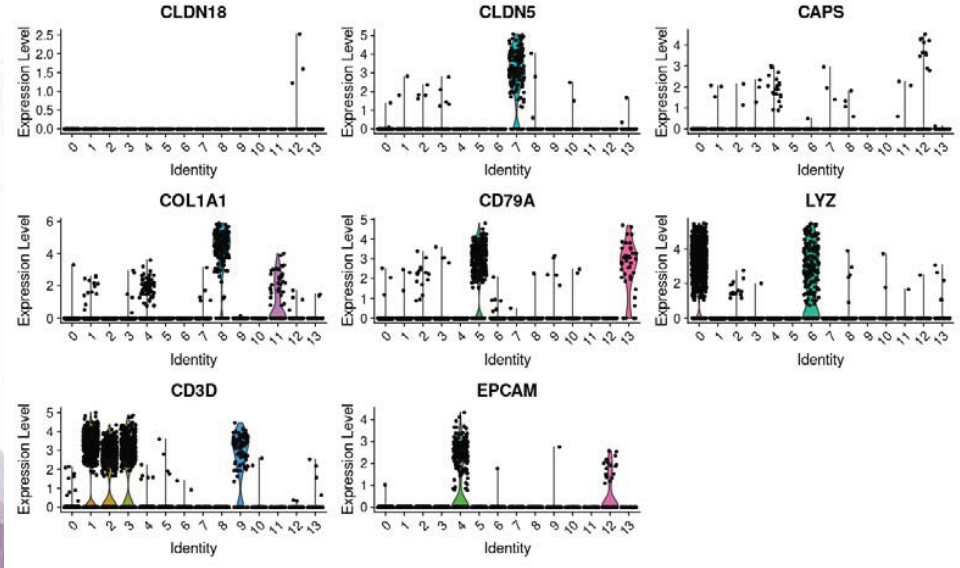


75

Lambrechts et al. Nature Medicine. 2018

Visualizing marker expression

- cell.markers <- c("CLDN18", "CLDN5", "CAPS", "COL1A1", "CD79A", "LYZ", "CD3D", "EPCAM")
- VlnPlot(object = seurat.obj, features = cell.markers)



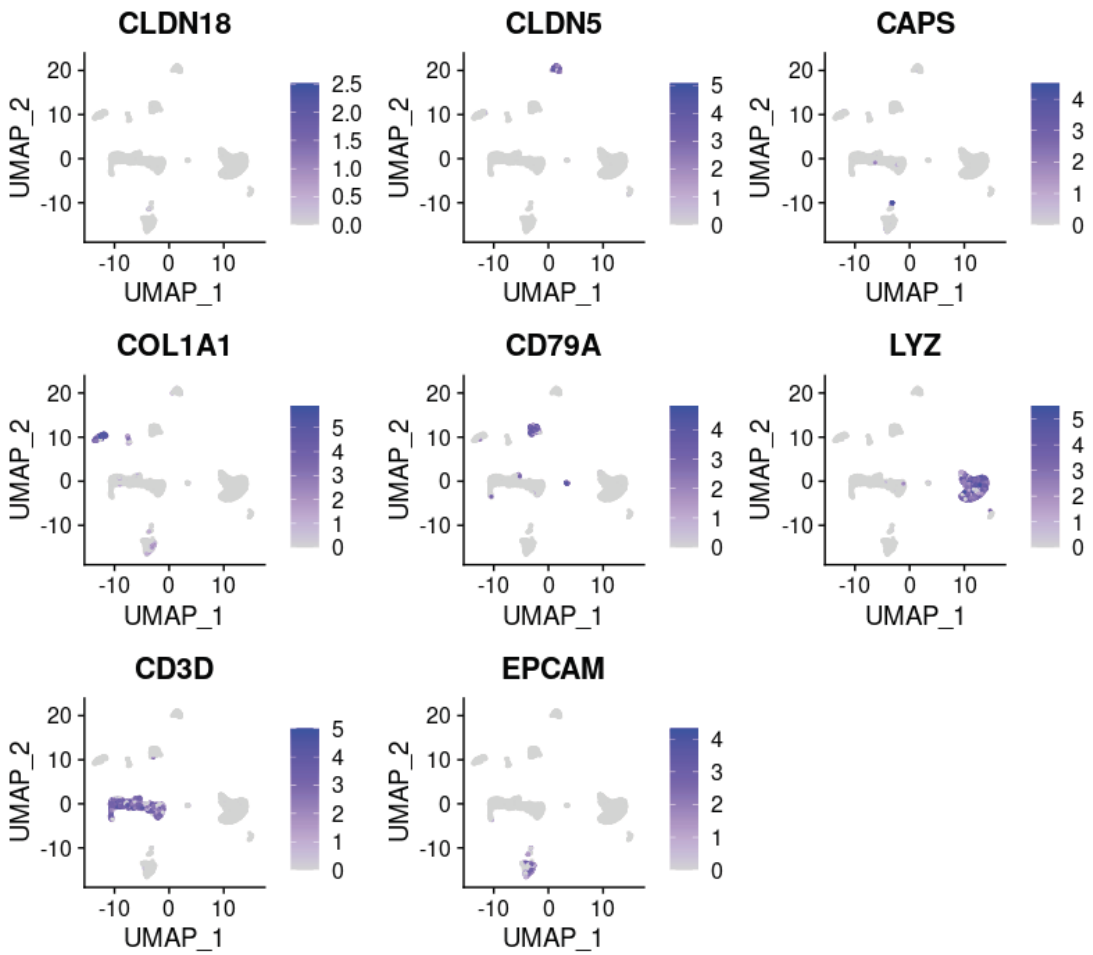
76

Visualizing marker expression

➢FeaturePlot(object = seurat.obj, reduction = "umap", features = cell.markers)

- ***features*** : Vector of features to plot
- ***reduction*** : Which dimensionality reduction to use. If not specified, first searches for *umap*, then *tsne*, then *pca*
- ***label*** : Whether to label the clusters

77



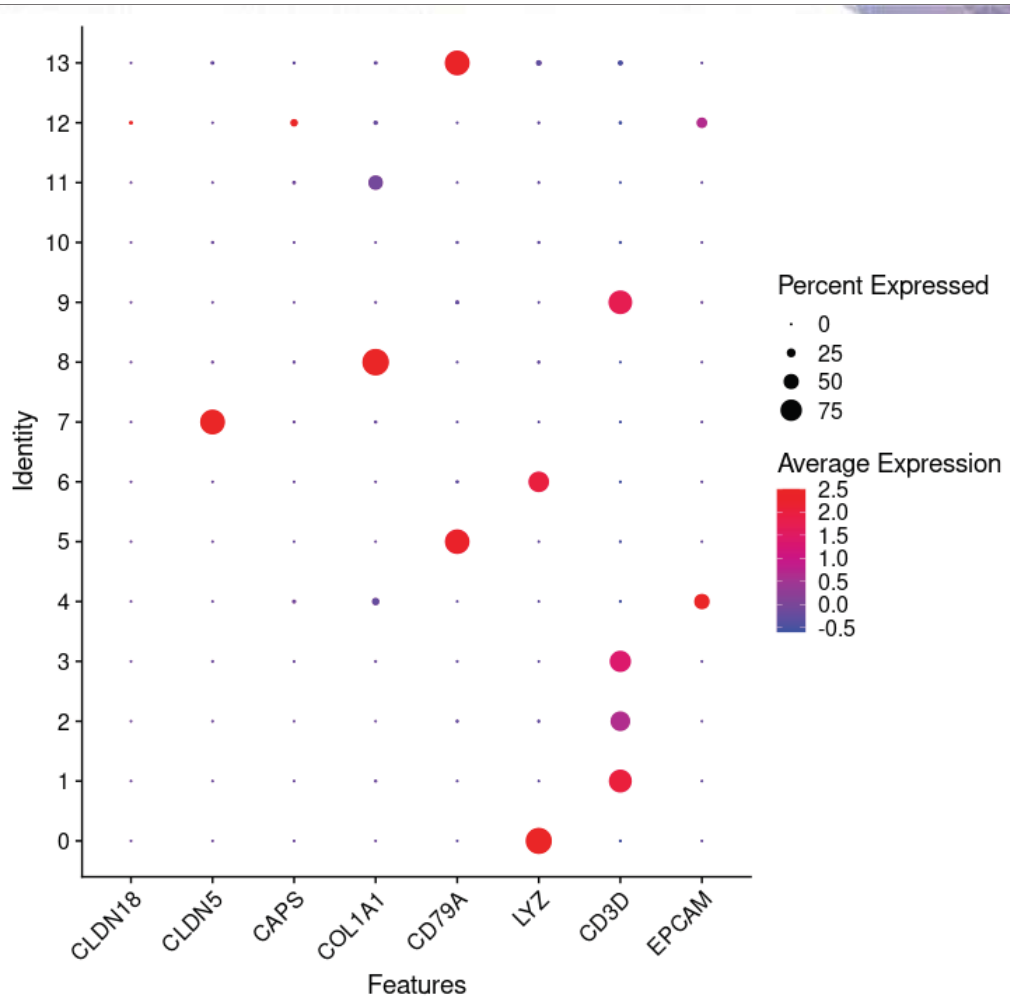
78

Visualizing marker expression

➢DotPlot(object = seurat.obj, features = cell.markers, cols = c("blue", "red")) + RotatedAxis()

- ***features*** : Input vector of features
- ***cols*** : Colors to plot, can pass a single character giving the name of a palette from *RColorBrewer::brewer.pal.info*
- ***group.by*** **:** Factor to group the cells by

79



80

Parallelization in Seurat with future

- library(future)
- options(future.globals.maxSize = 50000 * 1024^2)
- plan("multiprocess", workers = 10)
- plan()

NormalizeData, ScaleData, JackStraw, FindMarkers, FindIntegrationAnchors, FindClusters

81

Cell type annotation (SingleR)

https://bioconductor.org/packages/release/bioc/vignettes/SingleR/inst/doc/SingleR.html

82

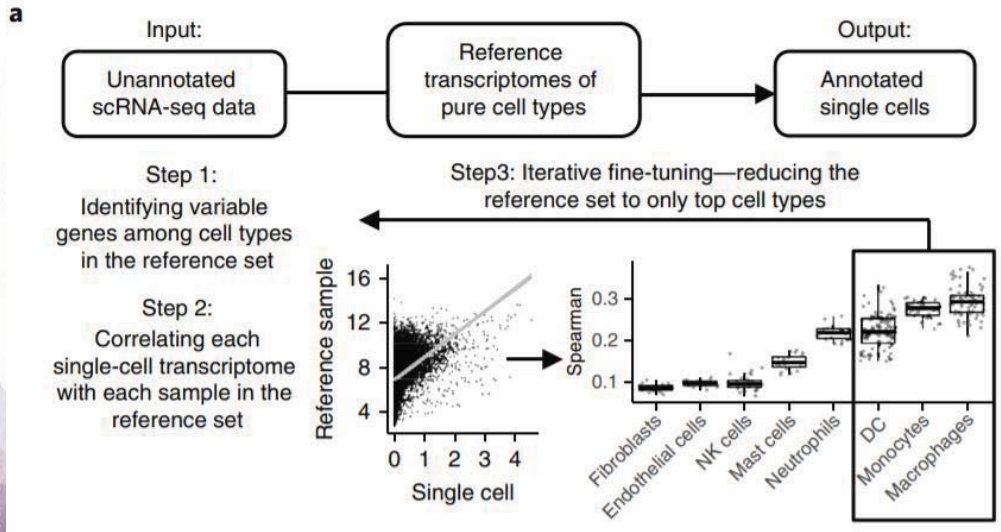
nature immunology

ARTICLES

https://doi.org/10.1038/s41590-018-0276-y

Reference-based analysis of lung single-cell sequencing reveals a transitional profibrotic macrophage

Dvir Aran[1,7], Agnieszka P. Looney[2,7], Leqian Liu[3,7], Esther Wu[2], Valerie Fong[2], Austin Hsu[4], Suzanna Chak[2], Ram P. Naikawadi[2], Paul J. Wolters[2], Adam R. Abate[3,5,6], Atul J. Butte[1] and Mallar Bhattacharya[2*]



83

Dvir Aran et al. Nature Immunology. 2019

Load data

- library(SingleR)
- library(Seurat)
- library(scater)
- seurat.obj <- readRDS(file = "~/BIML-2021-SingleCellRNAseq/Result/Seurat/Seurat.RDS")
- hpca.se <- HumanPrimaryCellAtlasData()

84

Available references

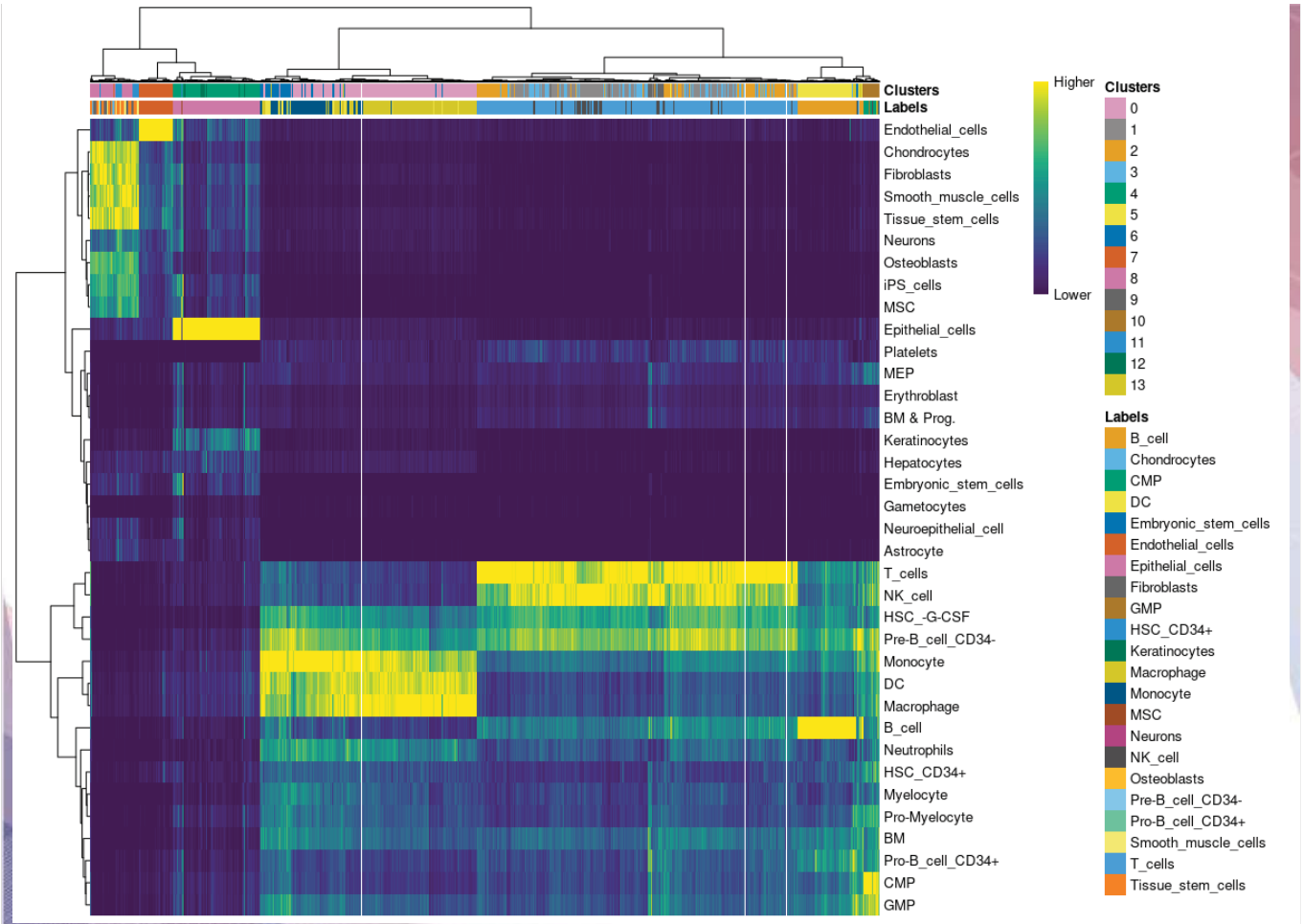
Data retrieval	Organism	Samples	Sample types	No. of main labels	No. of fine labels	Cell type focus
`HumanPrimaryCellAtlasData()`	human	713	microarrays of sorted cell populations	37	157	Non-specific
`BlueprintEncodeData()`	human	259	RNA-seq	24	43	Non-specific
`DatabaseImmuneCellExpressionData()`	human	1561	RNA-seq	5	15	Immune
`NovershternHematopoieticData()`	human	211	microarrays of sorted cell populations	17	38	Hematopoietic & Immune
`MonacoImmuneData()`	human	114	RNA-seq	11	29	Immune
`ImmGenData()`	mouse	830	microarrays of sorted cell populations	20	253	Hematopoietic & Immune
`MouseRNAseqData()`	mouse	358	RNA-seq	18	28	Non-specific

85

Annotate cell types

- luad.sc <- as.SingleCellExperiment(x = seurat.obj)
- luad.sc <- logNormCounts(luad.sc)
- pred.hesc <- SingleR(test = luad.sc, ref = hpca.se, labels = hpca.se$label.main)
- plotScoreHeatmap(pred.hesc, clusters = colData(luad.sc)$seurat_clusters)

86

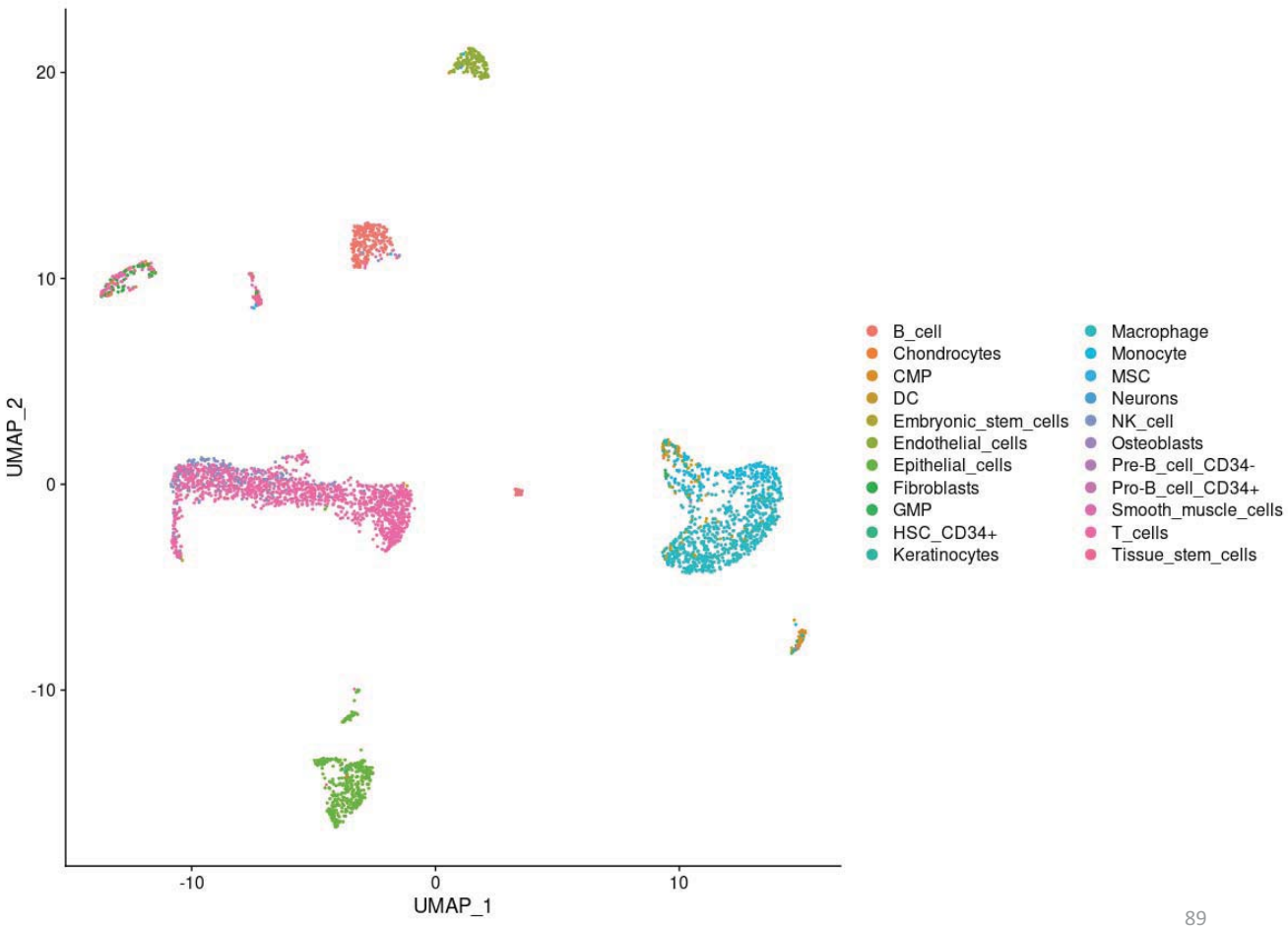


87

Annotate single-cell

- seurat.obj <- AddMetaData(object = seurat.obj, metadata = pred.hesc$labels, col.name = "SingleR.labels")
- DimPlot(object = seurat.obj, reduction = "umap", label = F, group.by = "SingleR.labels")

88



89

Annotate single-cell

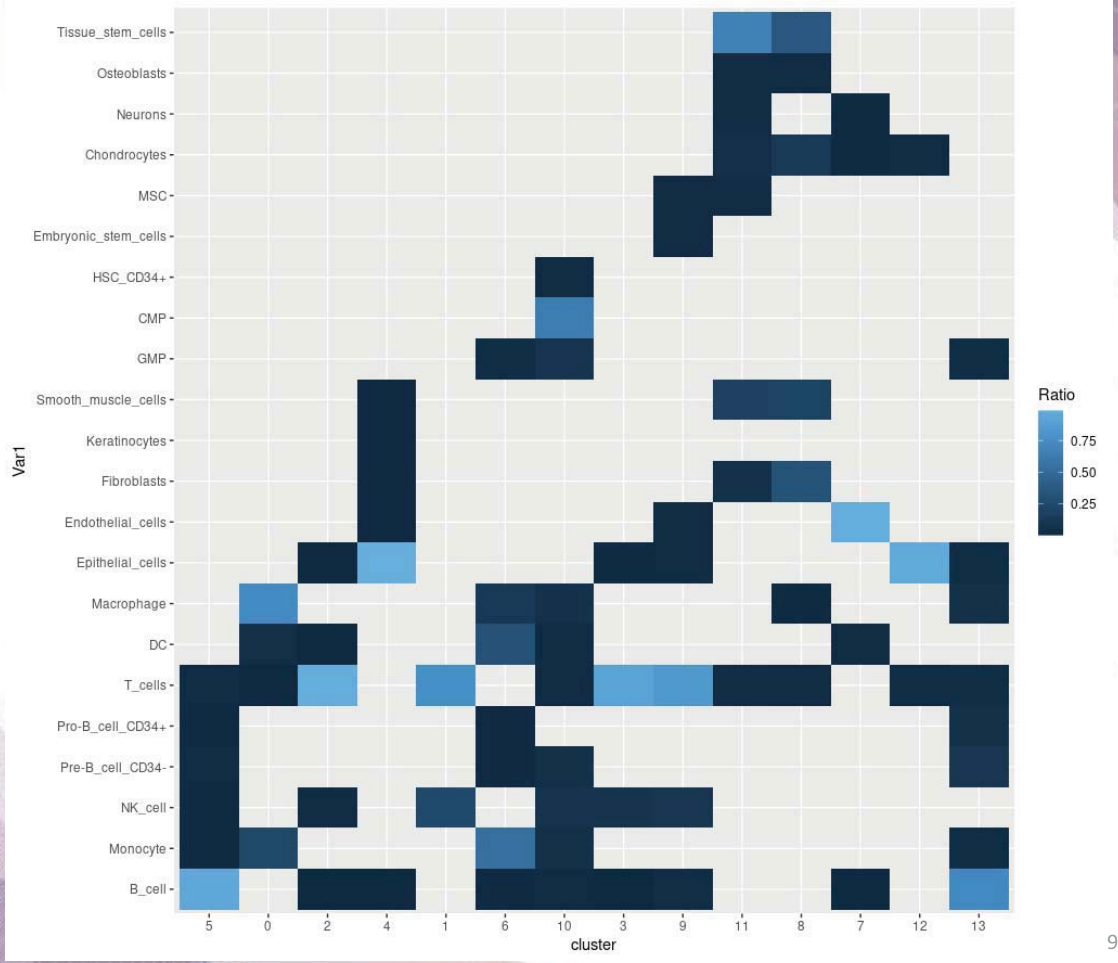
- count.df <- data.frame()
- for (temp.cluster in unique(seurat.obj@meta.data$seurat_clusters)){

```
temp.df <- data.frame(cluster = temp.cluster,
                      table(subset(seurat.obj@meta.data,
                      seurat_clusters ==
                      temp.cluster)$SingleR.labels))

temp.df$Ratio <- temp.df$Freq/sum(temp.df$Freq)

count.df <- rbind(count.df, temp.df)
```

}

- ggplot(data = count.df, aes(x = cluster, y = Var1, fill = Ratio)) + geom_tile()

90



91

Data Integration (Seuart)

https://satijalab.org/seurat/index.html

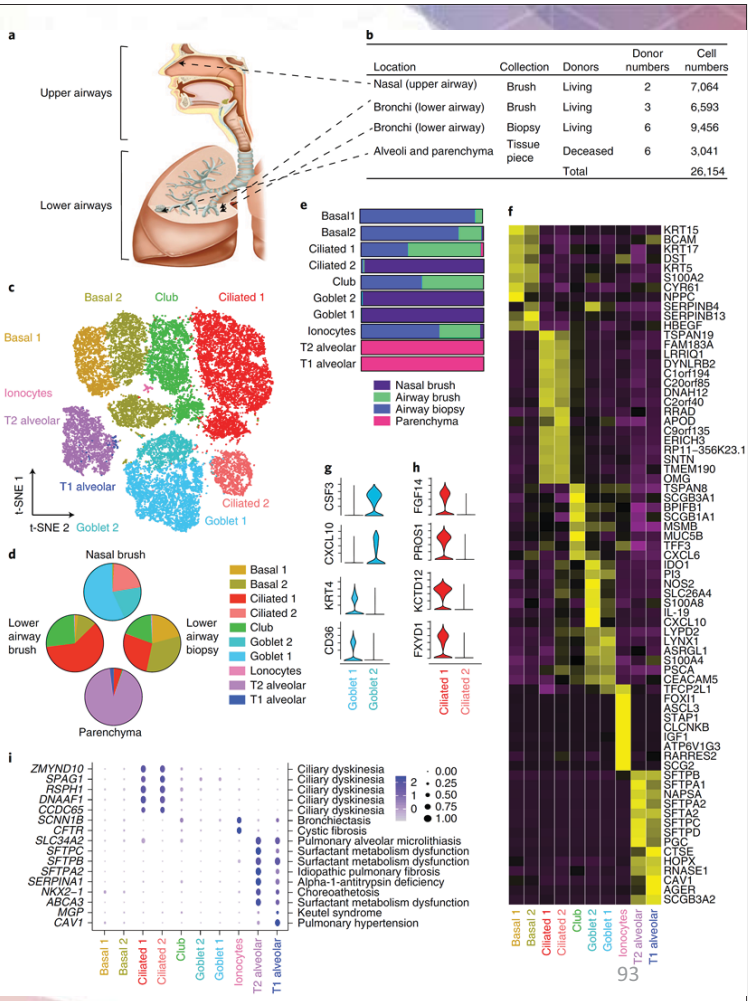
92

Reference dataset

nature medicine ARTICLES
https://doi.org/10.1038/s41591-019-0468-5

A cellular census of human lungs identifies novel cell states in health and in asthma

Felipe A. Vieira Braga[1,2,18], Gozde Kar[1,2,18], Marijn Berg[3,4,18], Orestes A. Carpaij[4,5,18], Krzysztof Polanski[1,18], Lukas M. Simon[6], Sharon Brouwer[3,4], Tomás Gomes[1], Laura Hesse[3,4], Jian Jiang[3,4], Eirini S. Fasouli[1,2], Mirjana Efremova[1], Roser Vento-Tormo[1], Carlos Talavera-López[1], Marnix R. Jonker[3,4], Karen Affleck[7], Subarna Palit[6,8,9,10], Paulina M. Strzelecka[1,11,12], Helen V. Firth[7], Krishnaa T. Mahbubani[13], Ana Cvejic[1,11,12], Kerstin B. Meyer[1], Kourosh Saeb-Parsy[13], Marjan Luinge[3,4], Corry-Anke Brandsma[3,4], Wim Timens[3,4], Ilias Angelidis[14], Maximilian Strunz[14], Gerard H. Koppelman[4,15], Antoon J. van Oosterhout[5], Herbert B. Schiller[14], Fabian J. Theis[6,16], Maarten van den Berge[4,5], Martijn C. Nawijn[3,4,19]* and Sarah A. Teichmann[1,2,17,19]*



Load data sets

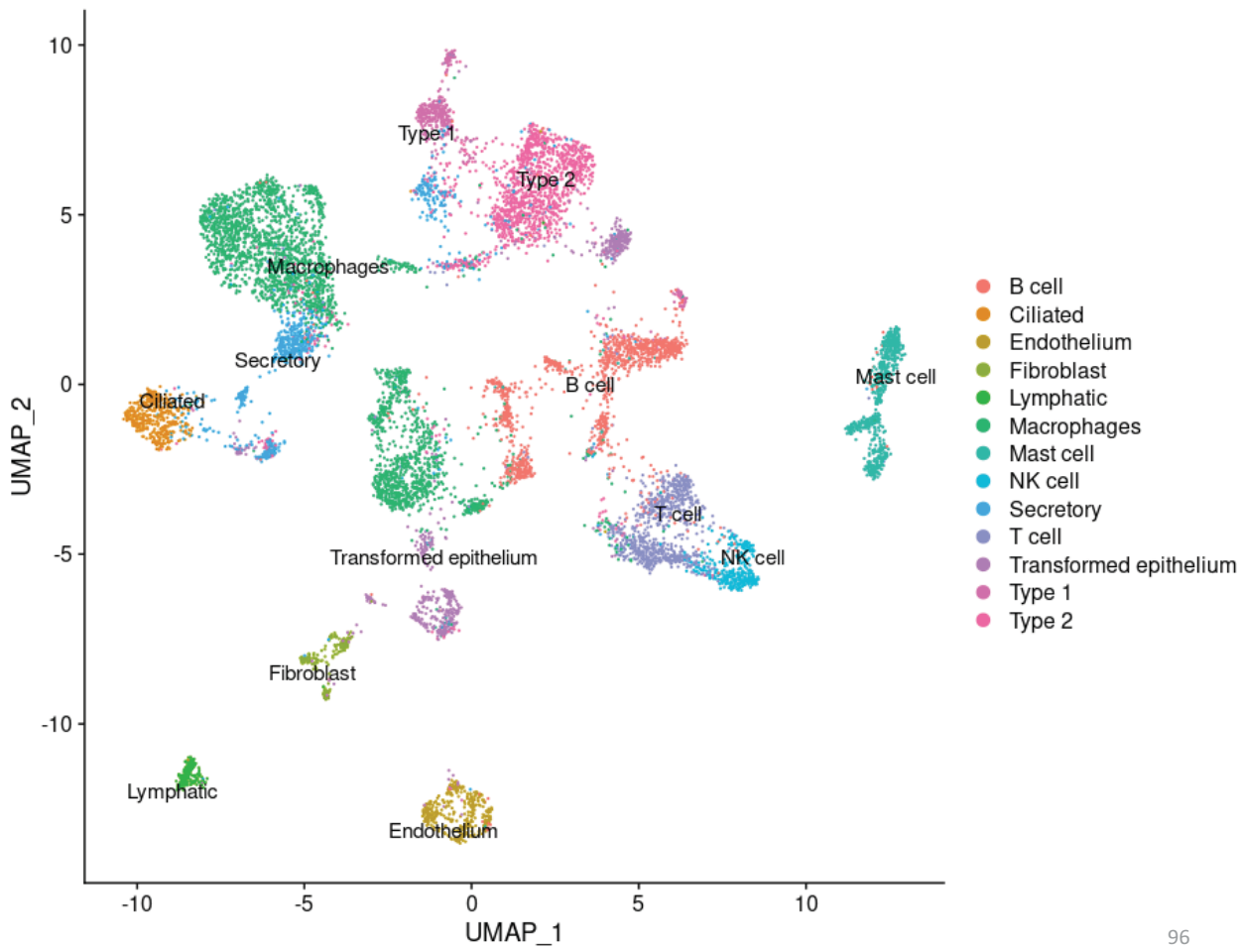
- library(Seurat)
- seurat.obj <- readRDS(file = "~/BIML-2021-SingleCellRNAseq/Result/Seurat/Seurat.result.RDS")
- sanger.count <- read.csv(file = "~/BIML-2021-SingleCellRNAseq/Resource/RawData/Sanger/GSE130148_raw_counts.csv", row.names = 1)
- sanger.meta <- read.table(file = "~/BIML-2021-SingleCellRNAseq/Resource/RawData/Sanger/GSE130148_barcodes_cell_types.txt", header = T, sep = "\t", row.names = 1)
- sanger.obj <- CreateSeuratObject(counts = sanger.count, meta.data = sanger.meta)

Clustering analysis

- sanger.obj <- NormalizeData(sanger.obj, verbose = F)
- sanger.obj <- FindVariableFeatures(sanger.obj, selection.method = "vst", nfeatures = 2000, verbose = F)
- sanger.obj <- ScaleData(sanger.obj, verbose = F)
- sanger.obj <- RunPCA(sanger.obj , npcs = 30, verbose = F)
- sanger.obj <- RunUMAP(sanger.obj, reduction = "pca", dims = 1:30, verbose = F)

- DimPlot(sanger.obj, reduction = "umap", group.by = "celltype", label = T, repel = T) + NoLegend()

95

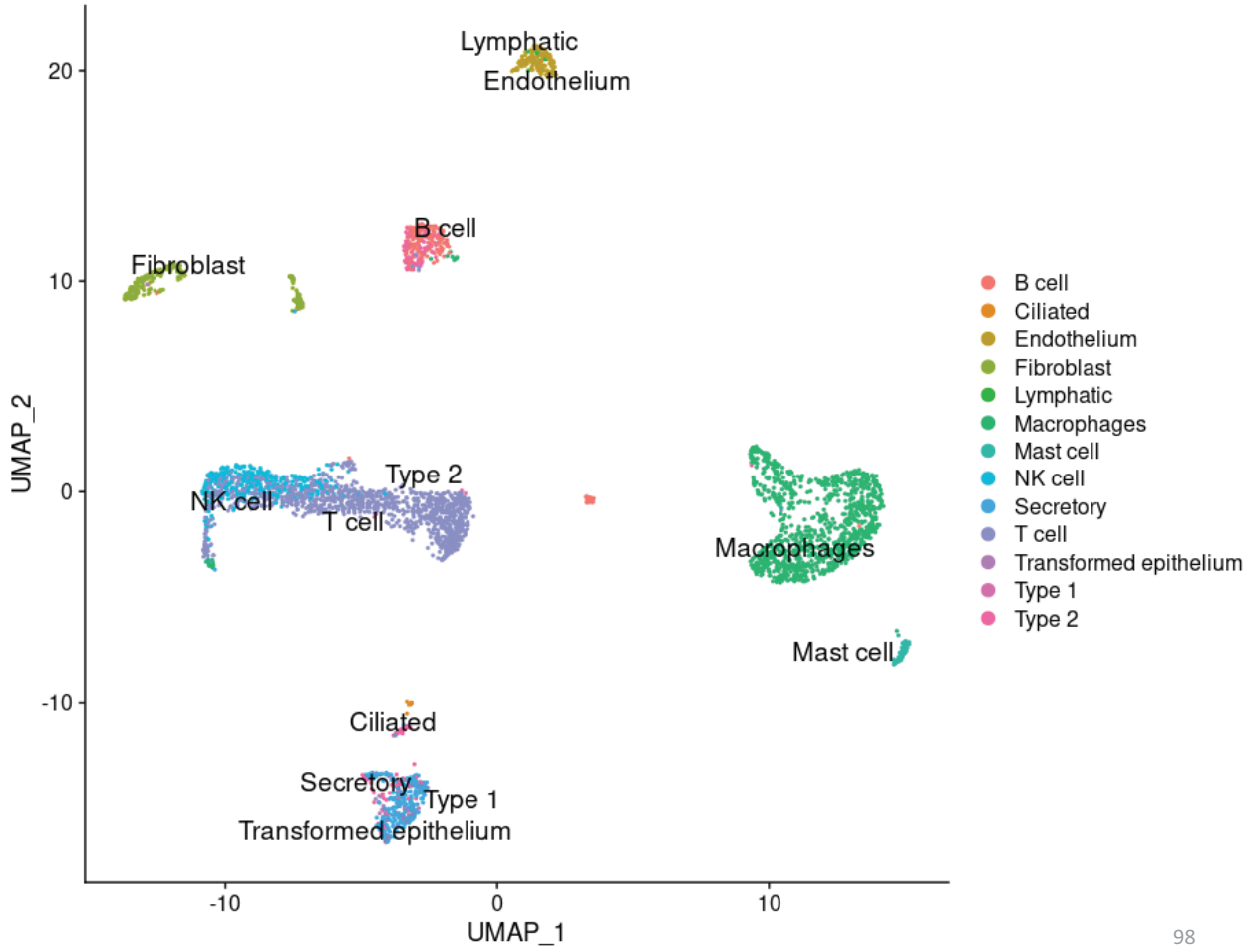


96

Integration

- anchors <- FindTransferAnchors(reference = sanger.obj, query = seurat.obj, dims = 1:30)
- predictions <- TransferData(anchorset = anchors, refdata = sanger.obj$celltype, dims = 1:30)
- query <- AddMetaData(seurat.obj, metadata = predictions)
- DimPlot(query, reduction = "umap", group.by = "predicted.id", label = T, label.size = 3, repel = T) + NoLegend()

97

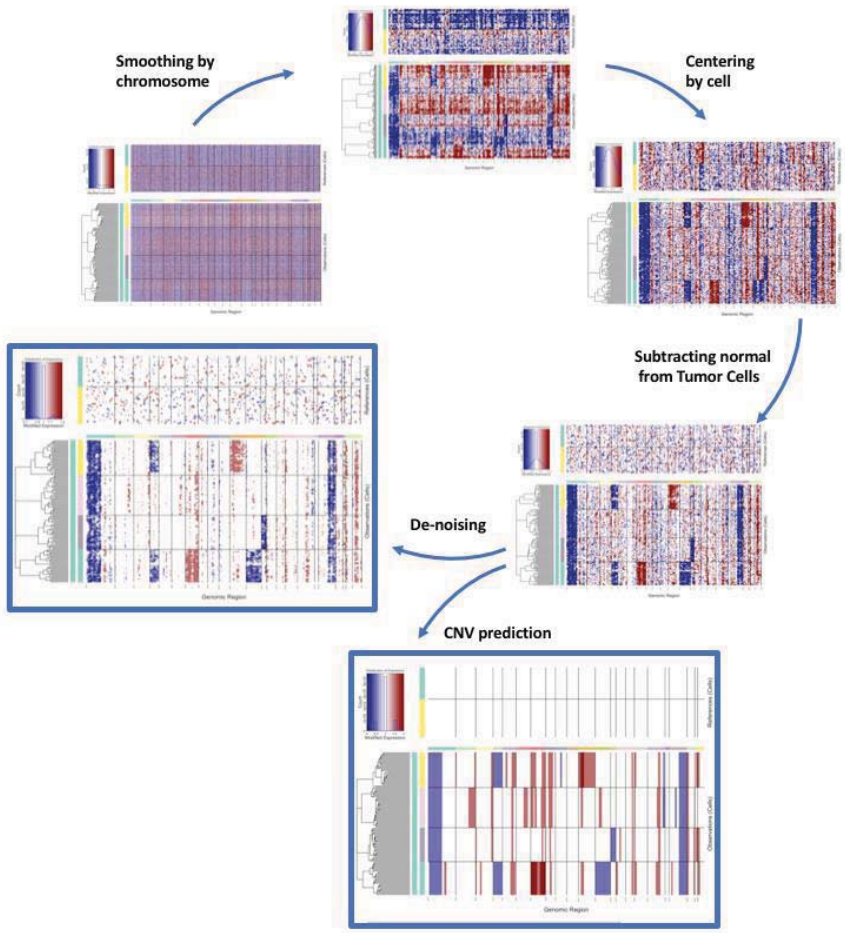


98

Inferring copy number alterations (InferCNV)

https://github.com/broadinstitute/inferCNV/wiki

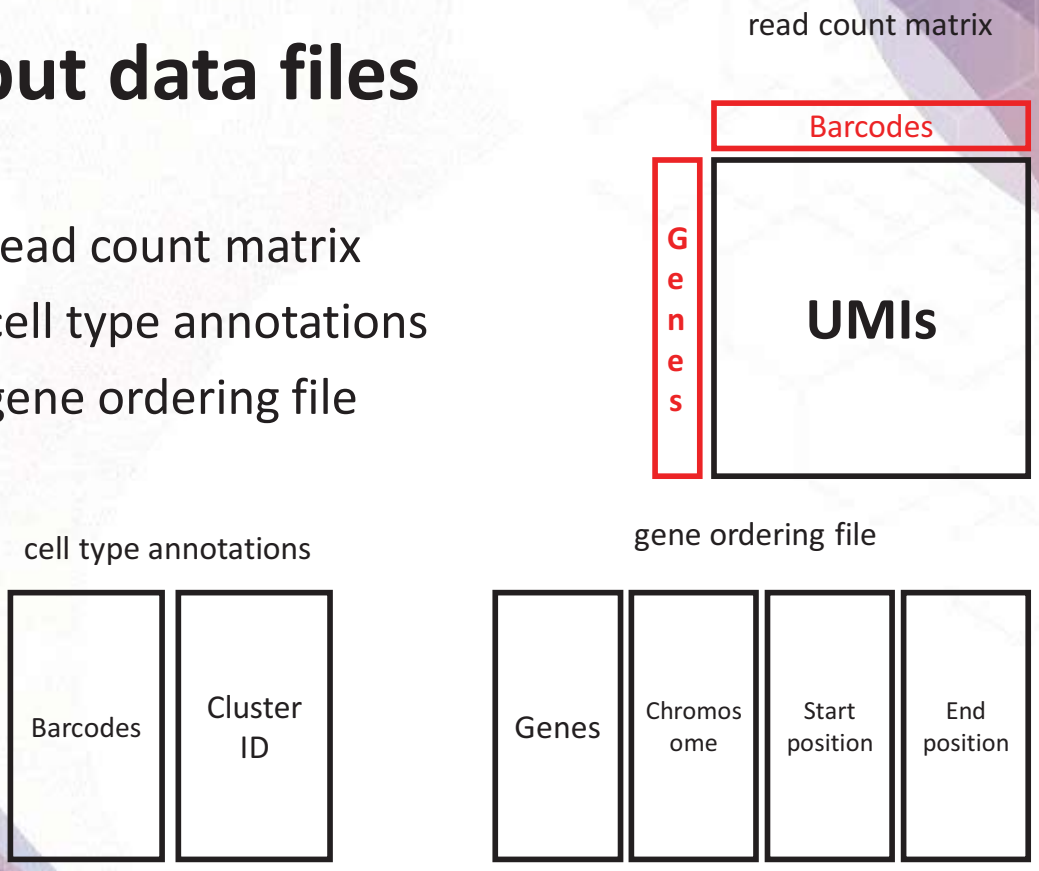
99



100

Input data files

1. read count matrix
2. cell type annotations
3. gene ordering file



101

Make input data

- library(Seurat)
- library(infercnv)
- seurat.obj <- readRDS(file = "~/BIML-2021-SingleCellRNAseq/Result/Seurat/Seurat.RDS")
- gene.order.file <- "~/BIML-2021-SingleCellRNAseq/Resource/Reference/hg19.inferCNV.gtf"

102

Make input data

- setwd("~/BIML-2021-SingleCellRNAseq/Result/inferCNV")
- write.table(x = as.matrix(seurat.obj@assays$RNA@counts), file = "./inferCNV.matrix", quote = F, sep = "\t")
- infer.cluster.df <- data.frame(id = rownames(seurat.obj@meta.data), cluster = paste0("C", seurat.obj@meta.data$seurat_clusters))
- write.table(x = infer.cluster.df, file = "./inferCNV.annotation", quote = F, sep = "\t", col.names = F, row.names = F)

Creating an InferCNV object

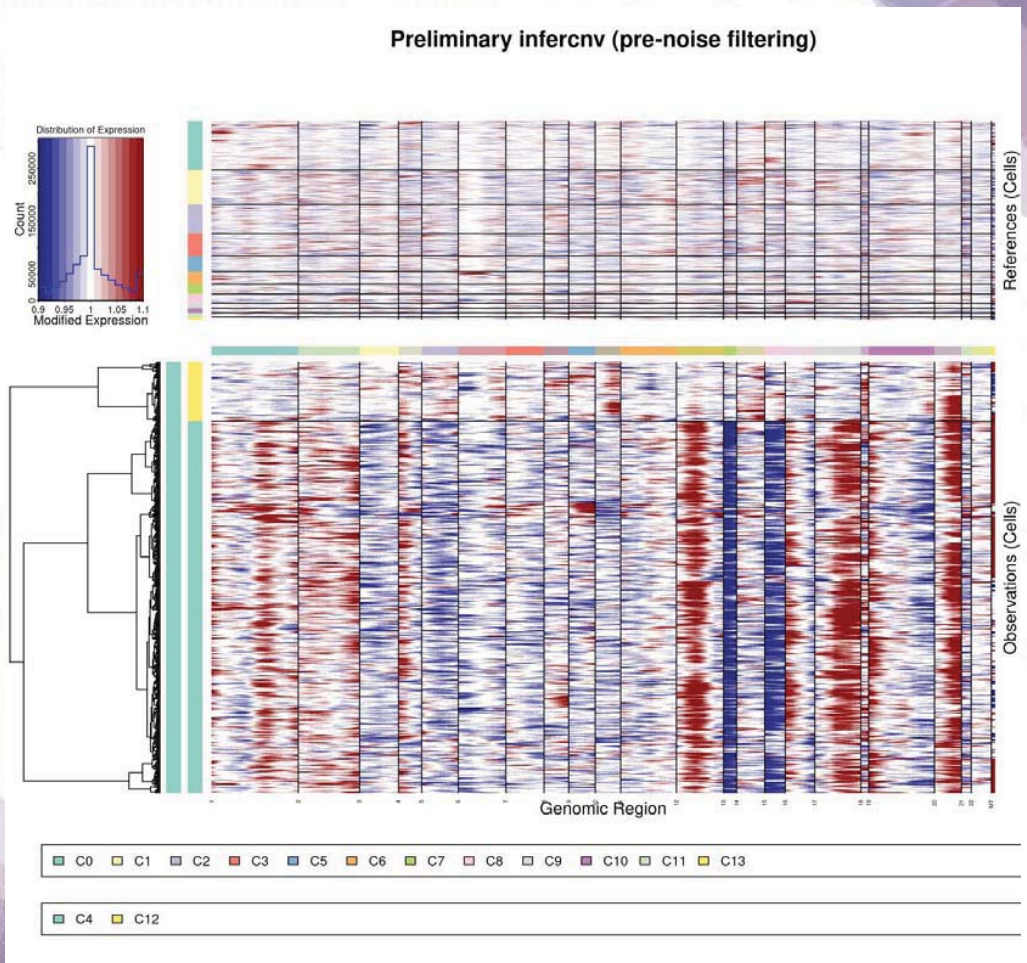
- ref.group <- c("C0", "C1", "C2", "C3", "C5", "C6", "C7", "C8", "C9", "C10", "C11", "C13")
  - Reference group is set to the various normal-cell type (non-tumor) as defined in the “cell type annotations” file
- infercnv.obj <- CreateInfercnvObject(raw_counts_matrix = "./inferCNV.matrix", annotations_file = "./inferCNV.annotation", gene_order_file = gene.order.file, ref_group_names = ref.group, delim = "\t", chr_exclude = c("X", "Y"))
  - ***delim*** **:** delimiter used in the input files
  - ***chr_exclude*** **:** list of chromosomes in the reference genome annotations that should be excluded from analysis (Default : c('chrX', 'chrY', 'chrM'))

Running InferCNV

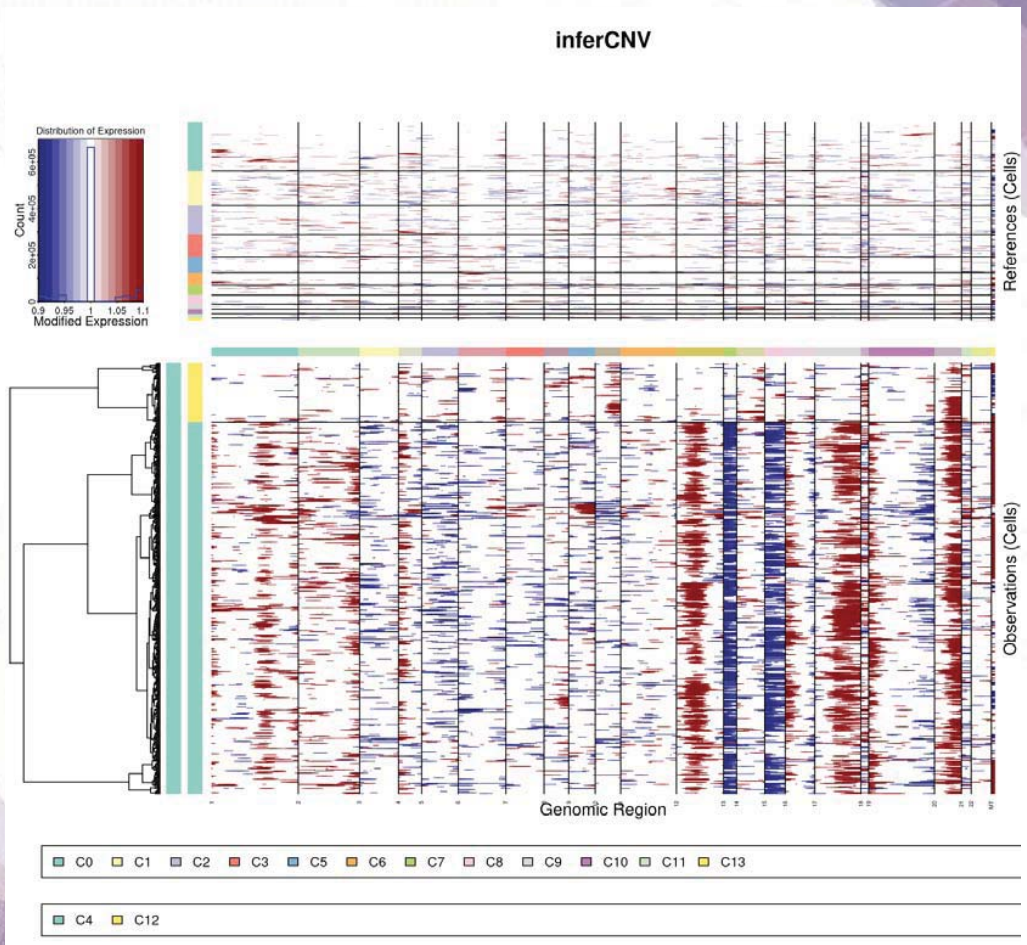
➢ infercnv.obj <- infercnv::run(infercnv_obj = infercnv.obj, cutoff = 0.1, cluster_by_groups = T, denoise = T, HMM = F, out_dir = "./")

- ***cutoff*** **:** Cut-off for the min average read counts per gene among reference cells. (default: 1)
  - ✓ use 1 for smart-seq, 0.1 for 10x-genomics
- ***cluster_by_groups*** **:** If observations are defined according to groups (ie. patients), each group of cells will be clustered separately. (default : FALSE)
- ***denoise*** **:** If True, turns on denoising according to options below
- ***HMM*** **:** when set to True, runs HMM to predict CNV level (default : FALSE)
  - ❖ This option can take a long time

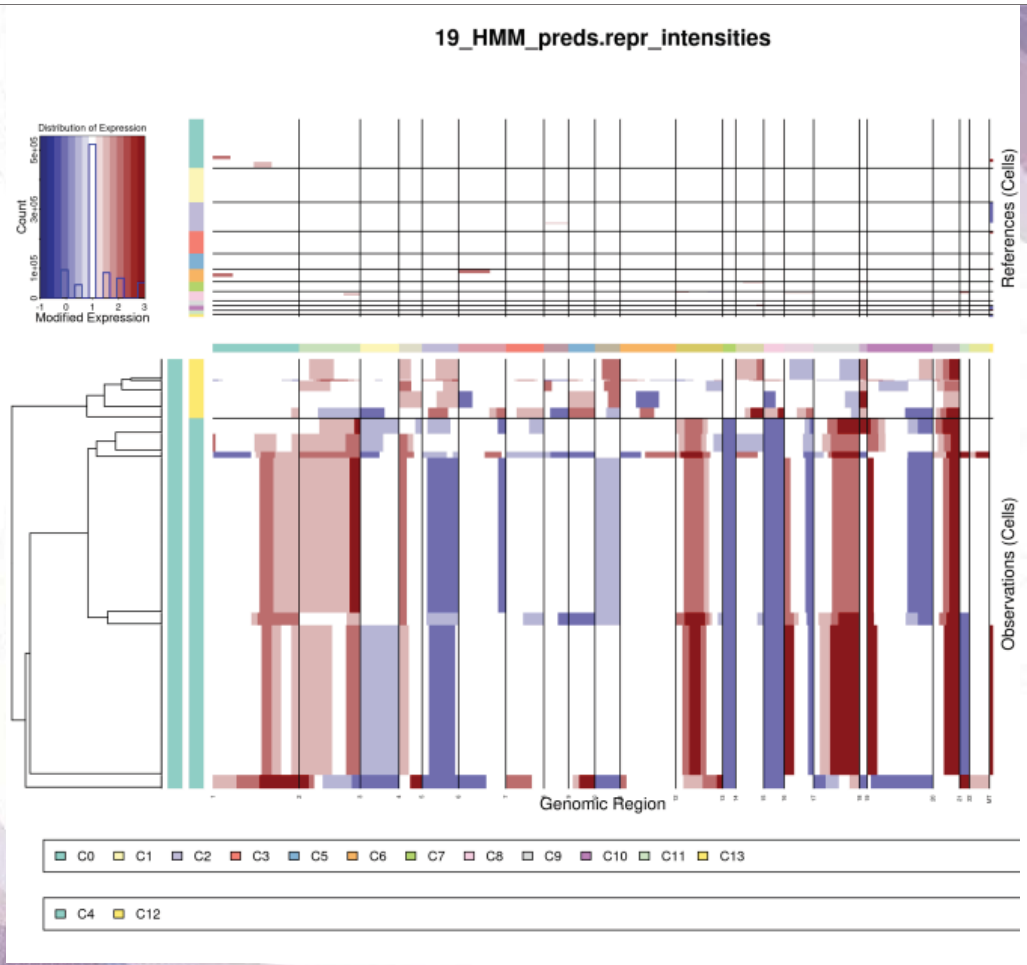
105



106


inferCNV
Distribution of Expression
Count
Modified Expression
References (Cells)
Observations (Cells)
Genomic Region
C0 C1 C2 C3 C5 C6 C7 C8 C9 C10 C11 C13
C4 C12

107

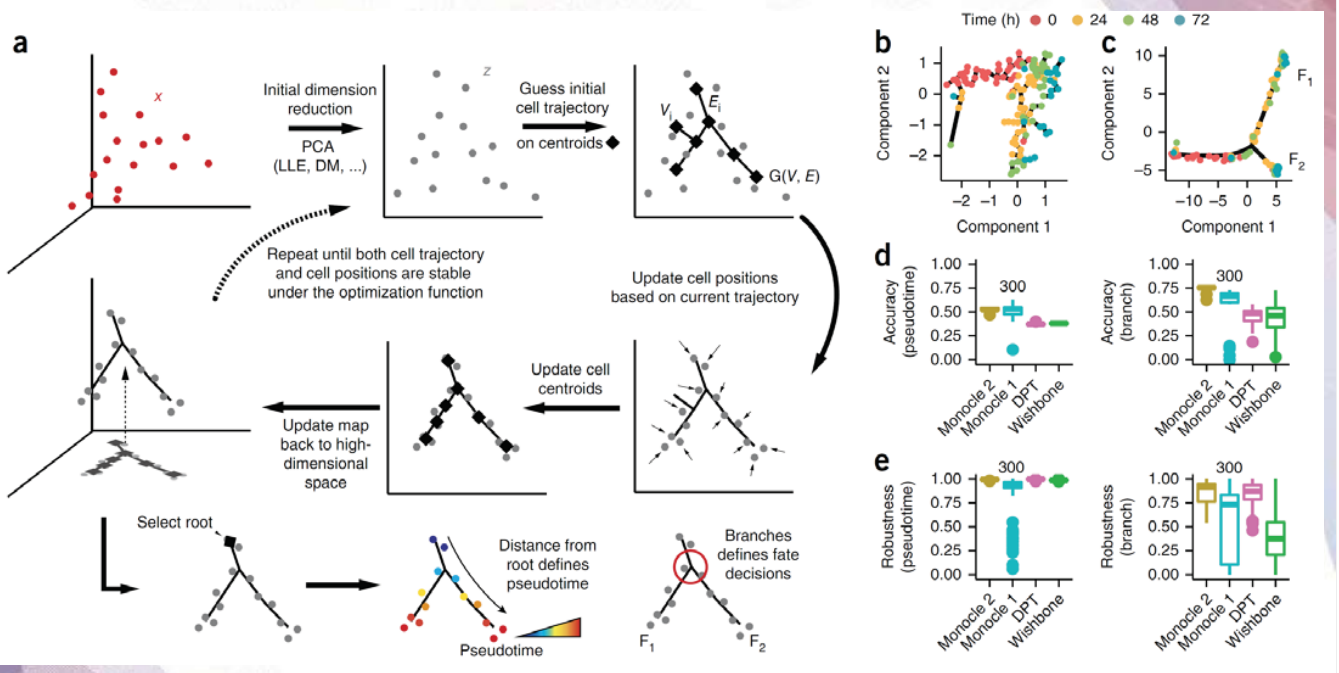

19_HMM_preds.repr_intensities
Distribution of Expression
Count
Modified Expression
References (Cells)
Observations (Cells)
Genomic Region
C0 C1 C2 C3 C5 C6 C7 C8 C9 C10 C11 C13
C4 C12

108

Trajectory analysis (monocle)

http://cole-trapnell-lab.github.io/monocle-release/docs/

109



Qiu et al. Nat Methods (2017)

110

T cell subclustering

- library(Seurat)
- library(monocle)
- seurat.obj <- readRDS(file = "~/BIML-2021-SingleCellRNAseq/Result/Seurat/Seurat.RDS")
- subset.obj <- subset(seurat.obj, seurat_clusters %in% c("1", "2", "3", "9"))

111

T cell subclustering

- subset.obj <- NormalizeData(object = subset.obj, normalization.method = "LogNormalize", scale.factor = 10000)
- subset.obj <- FindVariableFeatures(subset.obj, selection.method = "vst", nfeatures = 2000)
- subset.obj <- ScaleData(object = subset.obj, features = rownames(subset.obj), vars.to.regress = "percent.mt")
- subset.obj <- RunPCA(object = subset.obj, features = VariableFeatures(seurat.obj))

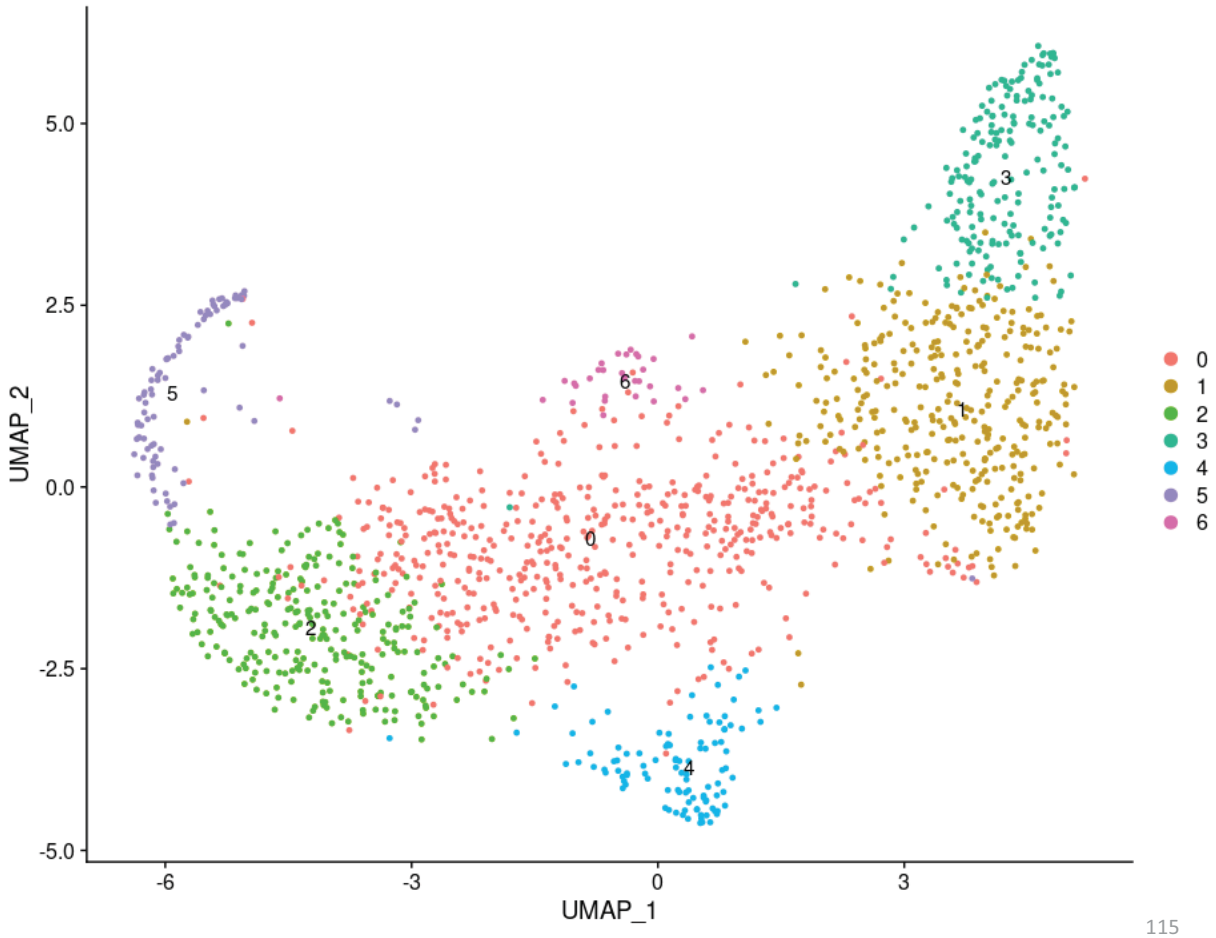
112

T cell subclustering

- subset.obj <- JackStraw(object = subset.obj, num.replicate = 100)
- subset.obj <- ScoreJackStraw(object = subset.obj, dims = 1:20)
- ElbowPlot(object = subset.obj)
- JackStrawPlot(object = subset.obj, dims = 1:20)

T cell subclustering

- subset.obj <- FindNeighbors(object = subset.obj, dims = 1:10)
- subset.obj <- FindClusters(object = subset.obj, resolution = 0.5)
- subset.obj <- RunUMAP(object = subset.obj, dims = 1:10)
- DimPlot(object = subset.obj, reduction = "umap", label = T)

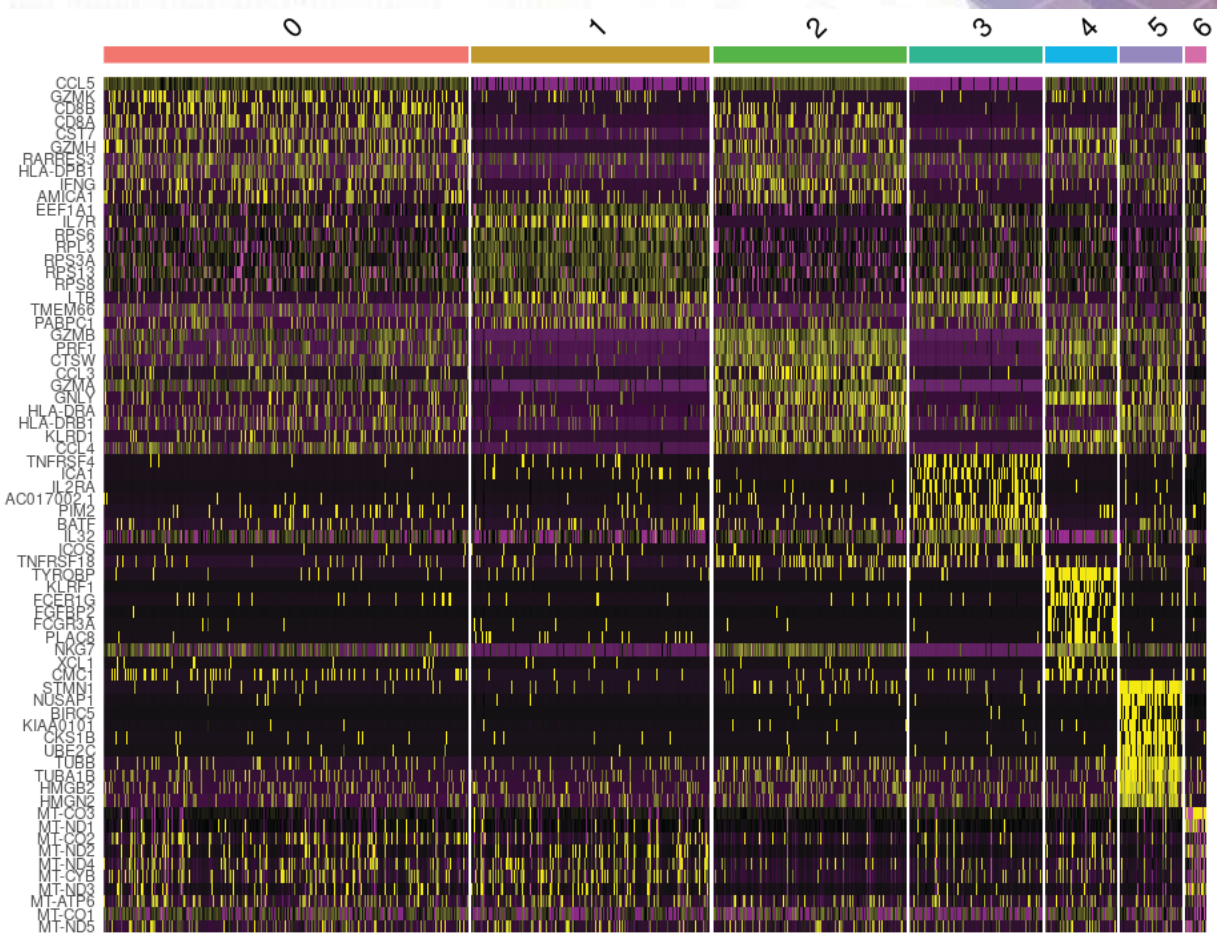


115

T cell subclustering

- subset.markers <- FindAllMarkers(object = subset.obj, only.pos = T, min.pct = 0.25, logfc.threshold = 0.25)
- subset.top.genes <- subset.markers %>% group_by(cluster) %>% top_n(n = 5, wt = avg_logFC)
- DoHeatmap(object = subset.obj, features = subset.top.genes$gene) + NoLegend()

116



117

Create monocle object

- data <- as(subset.obj@assays$RNA@data, "matrix")
- pd <- new('AnnotatedDataFrame', data = subset.obj@meta.data)
- gene.df <- data.frame(apply(X = data, 1, FUN = function(x) { sum(x > 0)}))
- fd <- new('AnnotatedDataFrame', data = data.frame(gene_short_name = row.names(gene.df), row.names = row.names(gene.df), num_cells_expressed = gene.df[,1]))
- cds <- newCellDataSet(data, phenoData = pd, featureData = fd, expressionFamily = negbinomial.size())

118

Estimate size factors and dispersions

- cds <- estimateSizeFactors(cds)
- cds <- estimateDispersions(cds)

119

Filtering low-quality cells

- set.seed(123)
- cds <- detectGenes(cds, min_expr = 0.1)
- expressed_genes <- row.names(subset(fData(cds), num_cells_expressed >= 50))
- cds <- cds[expressed_genes,]

120

Select genes by seurat

- subset.markers <- subset(subset.markers, p_val_adj < 0.05)
- subset.markers.top.genes <- subset.markers %>% group_by(cluster) %>% top_n(n = 50, wt = avg_logFC)
- order.genes <- unique(as.character(subset.markers.top.genes$gene))

121

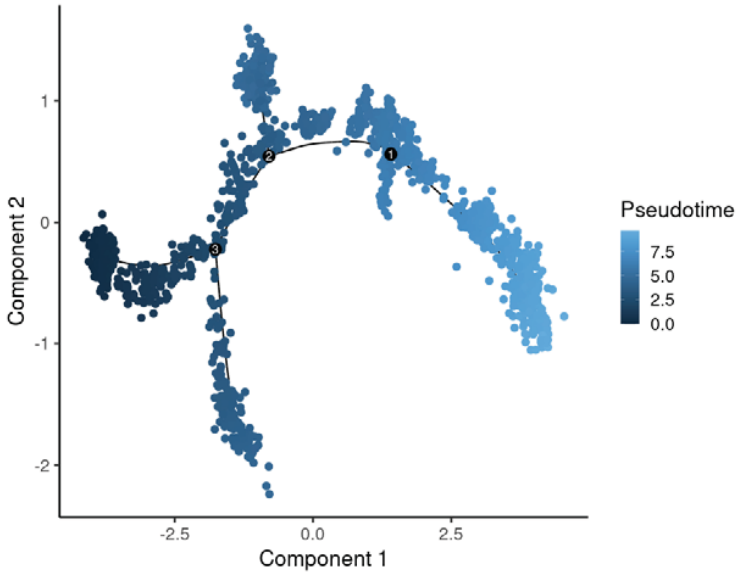
Order cells in pseudotime along a trajectory

- cds <- setOrderingFilter(cds, order.genes)
- cds <- reduceDimension(cds = cds, max_components = 3, method = 'DDRTree')
- cds <- orderCells(cds)

122

Plot trajectory

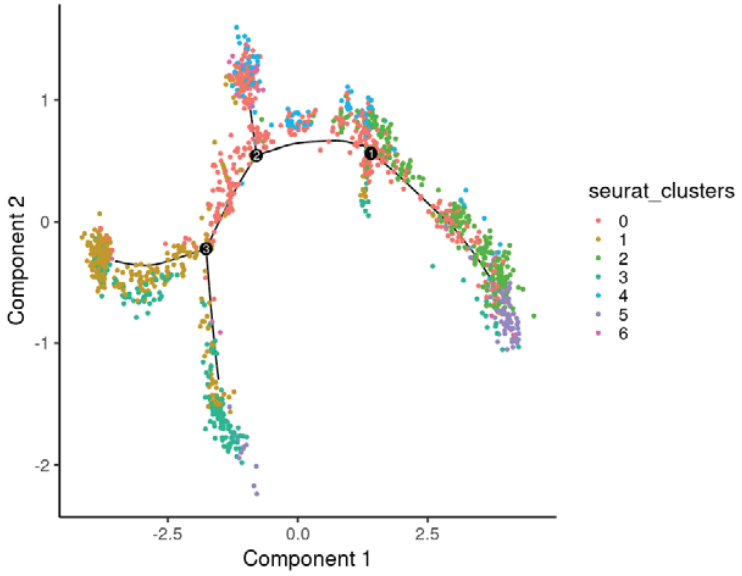
➢plot_cell_trajectory(cds, color_by = "Pseudotime", cell_size = 3) + theme_classic(base_size = 20)



123

Plot trajectory

➢plot_cell_trajectory(cds, color_by = "seurat_clusters") + theme_classic(base_size = 20)

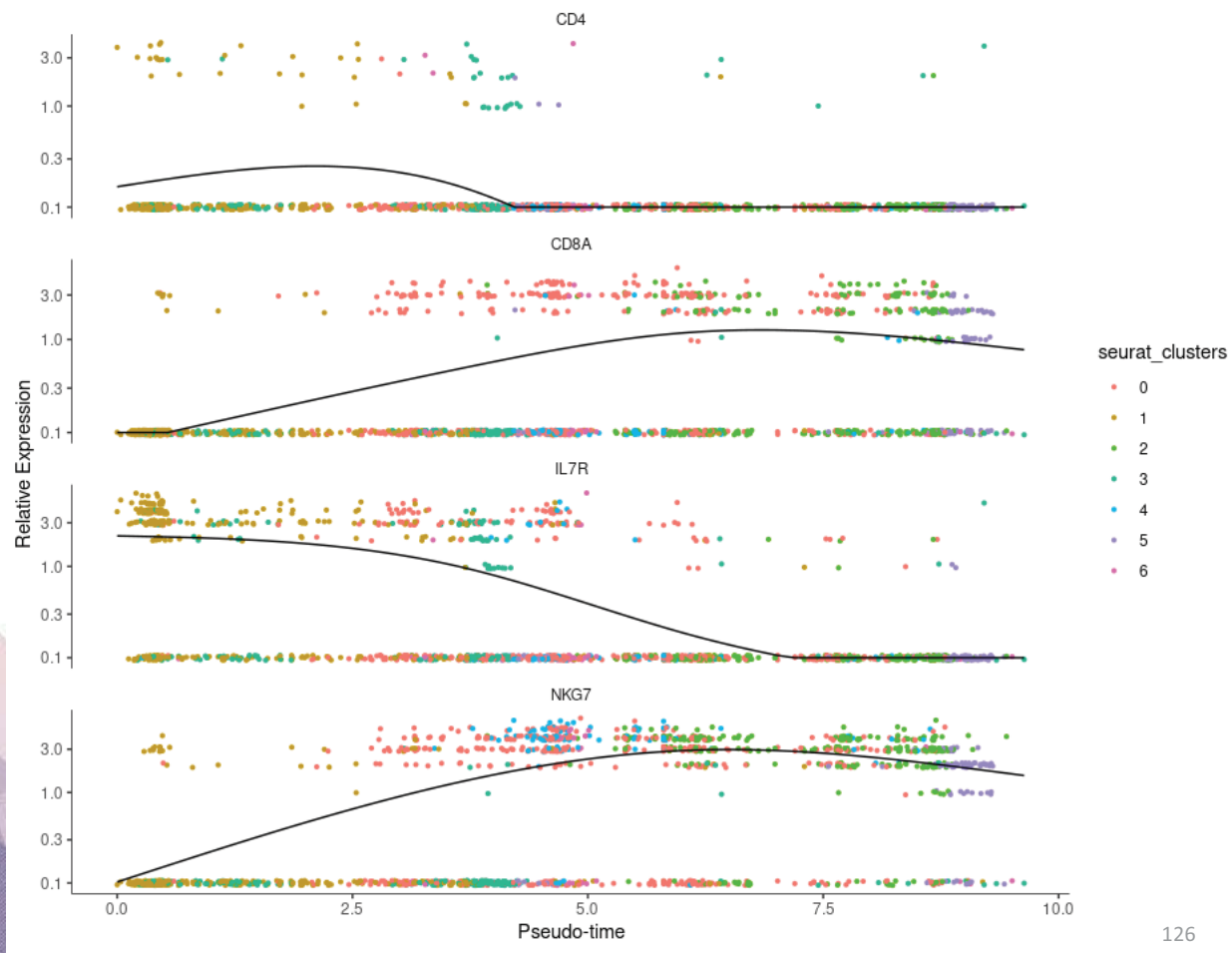


124

Plot trajectory

- my_genes <- row.names(subset(fData(cds), gene_short_name %in% c("IL7R", "CD4", "CD8A", "FOXP3", "NKG7")))
- cds_subset <- cds[my_genes,]
- plot_genes_in_pseudotime(cds_subset, color_by = "seurat_clusters")

125



126

KSBI | 한국생명정보학회

Thank you

Contact

Hyoung-oh Jeong

Email: hyoung-oh@unist.ac.kr