# KSBi-BIML 2023

Bioinformatics & Machine Learning(BIML)
Workshop for Life Scientists, Data Scientists,
and Bioinformatians

생물정보학&머신러닝 워크샵(온라인)

# Introduction to single cell transcriptomics analysis

김준일 _ 숭실대학교

SBi 한국생명정보학회
Korean Society for Bioinformatics

본 강의 자료는 한국생명정보학회가 주관하는 BIML 2023 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 2023

## Bioinformatics & Machine Learning (BIML)
## Workshop for Life Scientists, Data Scientists, and Bioinformatians

안녕하십니까?

한국생명정보학회가 개최하는 동계 교육 워크샵인 BIML-2023에 여러분을 초대합니다. 생명정보학 분야의 연구자들에게 최신 동향의 데이터 분석기술을 이론과 실습을 겸비해 전달하고자 도입한 전문 교육 프로그램인 BIML 워크샵은 2015년에 시작하여 올해로 9차를 맞이하게 되었습니다. 지난 2년간은 심각한 코로나 대유행으로 인해 아쉽게도 모든 강의가 온라인으로 진행되어 현장 강의에서만 가능한 강의자와 수강생 사이에 다양한 소통의 기회가 없음에 대한 아쉬움이 있었습니다. 다행히도 최근 사회적 거리두기 완화로 현장 강의가 가능해져 올해는 현장 강의를 재개함으로써 온라인과 현장 강의의 장점을 모두 갖춘 프로그램을 구성할 수 있게 되었습니다.

BIML 워크샵은 전통적으로 크게 인공지능과 생명정보분석 두 개의 분야로 구성되었습니다. 올해 AI 분야에서는 최근 생명정보 분석에서도 응용이 확대되고 있는 다양한 심층학습(Deep learning) 기법들에 대한 현장 강의가 진행될 예정이며, 관련하여 심층학습을 이용한 단백질구조예측, 유전체 분석, 신약개발에 대한 이론과 실습 강의가 함께 제공될 예정입니다. 또한 싱글셀오믹스 분석과 메타유전체분석 현장 강의는 많은 연구자의 연구 수월성 확보에 큰 도움을 줄 것으로 기대하고 있습니다. 이외에 다양한 생명정보학 분야에 대하여 30개 이상의 온라인 강좌가 개설되어 제공되며 온라인 강의의 한계를 극복하기 위해서 실시간 Q&A 세션 또한 마련했습니다. 특히 BIML은 각 분야 국내 최고 전문가들의 강의로 구성되어 해당 분야의 기초부터 최신 연구 동향까지 포함하는 수준 높은 내용의 강의가 될 것입니다.

이번 BIML-2023을 준비하기까지 너무나 많은 수고를 해주신 BIML-2023 운영위원회의 남진우, 우현구, 백대현, 정성원, 정인경, 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 연사분께 깊은 감사를 드립니다.

2023년 2월

**한국생명정보학회장 이 인 석**

## 강의개요

# Introduction to single cell transcriptomics analysis

2009년 단일세포에서 얻은 RNA sequencing 데이터가 처음 발표된 이후로 이제는 single cell RNA sequencing (scRNAseq)은 유전자발현 연구에 필수적인 도구로 자리잡고 있다. 이에 따라 데이터분석의 기본적인 파이프라인도 표준화되고 있지만 여전히 처음 접하는 연구자에게는 접근하기에 많은 어려움이 있다.

본 강의에서는 단일세포기술의 역사와 기본분석 파이프라인에 대해서 공부하고 가장 널리 쓰이는 Seurat과 Scanpy 사용법에 대해 설명한다. 이론강의에서 분석파이프라인이 만들어진 배경에 대해서 학습하고 그 원리를 탐구한 후에 예제 데이터를 통하여 Seurat과 Scanpy의 구성과 활용법에 대해서 익히고 연구자들이 생산한 데이터에 적용하여 분석하고 해석할 수 있는 역량을 갖추는 것을 목표로 한다.

강의는 다음의 내용을 포함한다:

- 단일세포 기술의 역사
- scRNAseq 데이터분석 기본 파이프라인 (품질관리, 정규화, 차원축소, 시각화, 클러스터링)
- Seurat를 활용한 scRNAseq 데이터 분석
- Scanpy를 활용한 scRNAseq 데이터 분석

* 참고강의교재:

  Current best practices in single-cell RNA-seq analysis: a tutorial / Malte Luecken, Fabian Theis

* 교육생준비물:

  Seurat (R package), Scanpy (Python package)가 설치된 노트북 (메모리 16GB 이상)

* 강의 난이도: 중급

* 강의: 김준일교수 (숭실대학교 컴퓨터공학부)

# Curriculum Vitae

## Speaker Name: Junil Kim, Ph.D.


▶ **Personal Info**

| | |
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| Name | Junil Kim |
| Title | Assistant Professor |
| Affiliation | Soongsil University |

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**Research Interest**

Single Cell Genomics, Systems Biology, Network Biology

**Educational Experience**

| | |
|---|---|
| 2005 | B.S. in Bioinformatics, Soongsil University, Republic of Korea |
| 2008 | M.S. in Bioinformatics, Seoul National University, Republic of Korea |
| 2014 | Ph.D. in Bio and Brain Engineering, KAIST, Republic of Korea |

**Professional Experience**

| | |
|---|---|
| 2014-2016 | Postdoctoral Researcher, CHA Cancer Institute, CHA University, Republic of Korea |
| 2016-2018 | Postdoctoral Researcher, Perelman School of Medicine, University of Pennsylvania, USA |
| 2018-2021 | Postdoctoral Researcher, BRIC, University of Copenhagen, Denmark |
| 2021- | Assistant Professor, School of Systems Biomedical Science, Soongsil University, Republic of Korea |

**Selected Publications (5 maximum)**


1. Guangzheng Weng, **Junil Kim***, and Kyoung Jae Won*, "VeTra: a tool for trajectory inference based on RNA velocity", Bioinformatics (**IF: 6.937**), btab364, May 2021. (*Co-corresponding authors)
2. **Junil Kim**, Simon T. Jakobsen, Kedar N. Natarajan, Kyoung Jae Won, "TENET: gene network reconstruction using transfer entropy reveals key regulatory factors from single cell transcriptomic data", Nucleic Acids Research (**IF: 16.971**), Vol. 49, No. 1, e1-e1, Jan. 2021.
3. Shibiao Wan, **Junil Kim**, Kyoung Jae Won, "SHARP: hyper-fast and accurate processing of single-cell RNA-seq data via ensemble random projection", Genome Research (IF: 9.043), Vol. 30, Issue 2, 205-213, Jan. 2020. (Google Scholar Citations: **11** / Web of Science Citations: **4**)
4. **Junil Kim**, Diana E. Stanescu, and Kyoung Jae Won, "CellBIC: Bimodality-based top-down clustering of single-cell RNA sequencing data reveals hierarchical structure of the cell type", Nucleic Acids Research (**IF: 16.971**), Vol. 46, Issue 21, e124, Aug. 2018. (Google Scholar Citations: **8** / Web of Science Citations: **4**)
5. **Junil Kim**, Sang-Min Park, and Kwang-Hyun Cho, "Discovery of a kernel for controlling biomolecular regulatory networks", Scientific Reports, Vol. 3, 2223, July 2013. (Google Scholar Citations: **87** / Web of Science Citations: **44**)

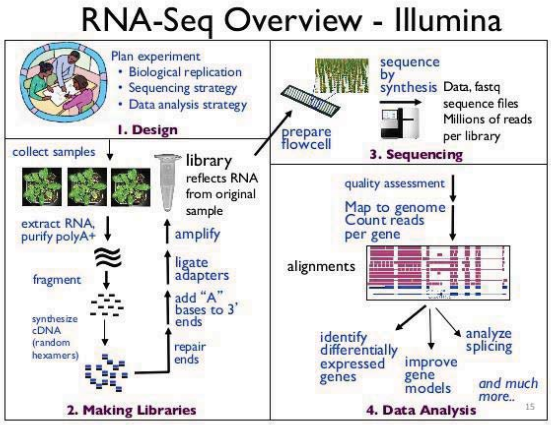
# KSBi-BIML

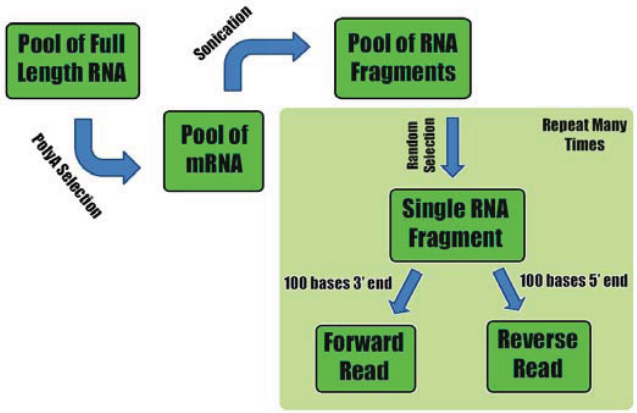
Introduction to single cell transcriptomics analysis

## Contents


1. Review of Bulk RNA sequencing
2. Why single cell?
3. Single cell technology
4. What can we do using single cell transcriptomics?
   - Dimension reduction and clustering (cell type identification)
   - Dynamics (Pseudotime, RNA velocity, GRN analysis)
   - Beyond single cell (Cell-cell interaction, Spatial transcriptomics)
5. Single cell transcriptomics analysis pipeline
   - Quality control and normalization
   - Feature selection
   - Dimension reduction and visualization
   - Clustering and cell type annotation

# 1. Review of Bulk RNA sequencing: mRNA sequencing exactly measures the quantity of mRNA molecule




- *Full length* mRNA cannot yet be sequenced routinely (Illumina).
  - Only short fragments can be sequenced accurately and cheaply.
- RNA are fragmented into small pieces, typically 200 - 500 bases.
- Approximately **100 bases** are sequenced from one, or both, ends of the fragments.

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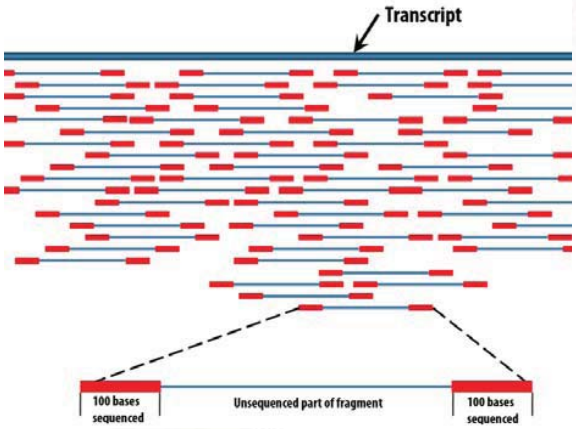
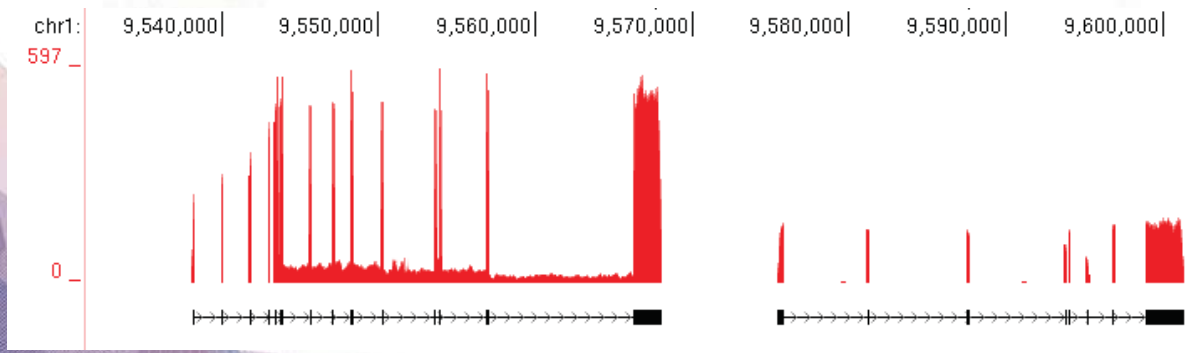
# 1. Review of Bulk RNA sequencing




- Reads are aligned to the genome.
- Data are represented as "depth of coverage" plots.
  - The height of the bar over a nucleotide is the number of reads which align across that location.
- The higher a gene is expressed, the more reads we find for that gene.
- The higher the peak, the higher the gene is expressed.

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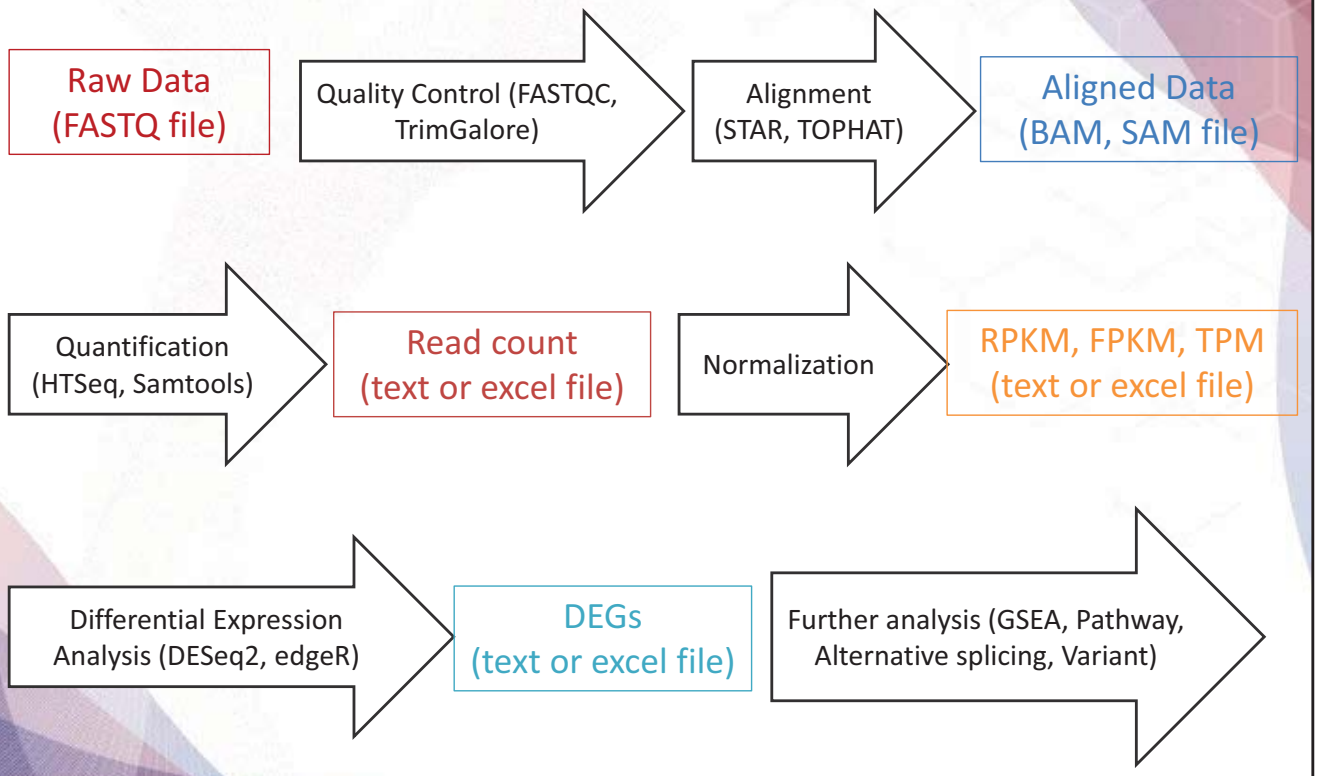
## 1. Review of Bulk RNA sequencing: RNA-seq analysis pipeline

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## 2. Why single cell?

- Cell identity and function can be characterized at the molecular level by unique transcriptome signatures (Stegle et al. Nature Reviews Genetics 2015)
- Ensemble-based approaches (bulk RNA-seq) only provide an average of each gene's expression across a large population of cells.
- What we cannot see using bulk measures?
  1. Heterogeneity – early embryo, complex tissues such as brain tissues
  2. Cellular composition – Differential gene expression between samples may be driven by cellular composition
  3. Stochasticity – gene expression bursts probabilistically

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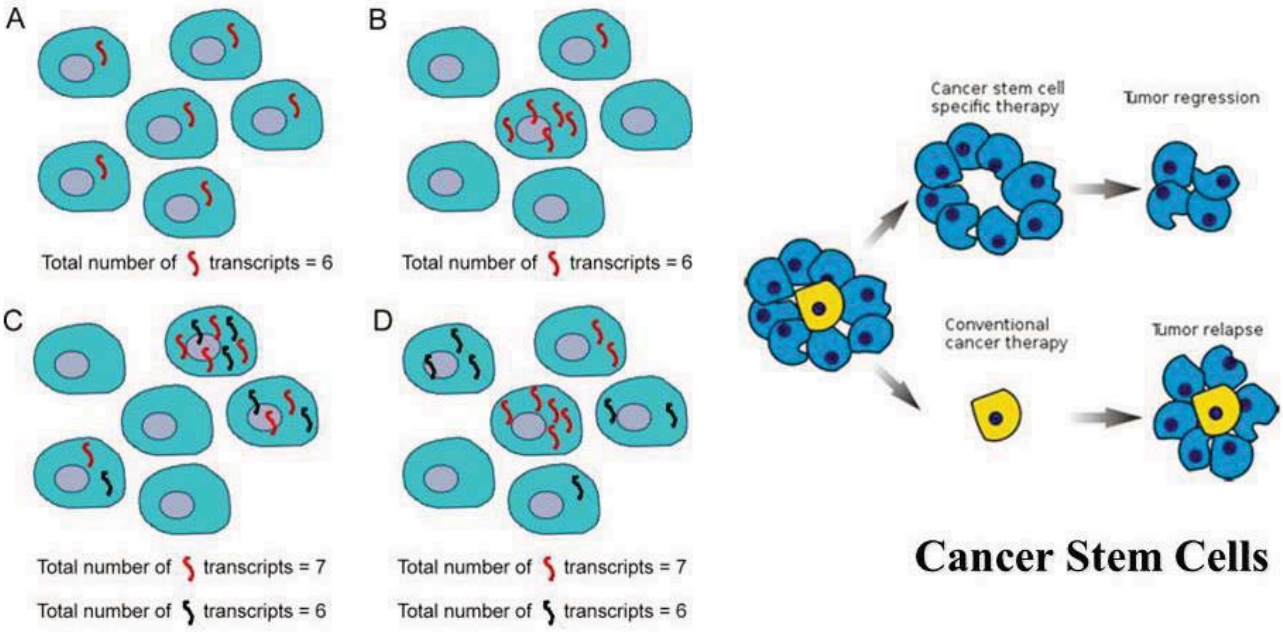
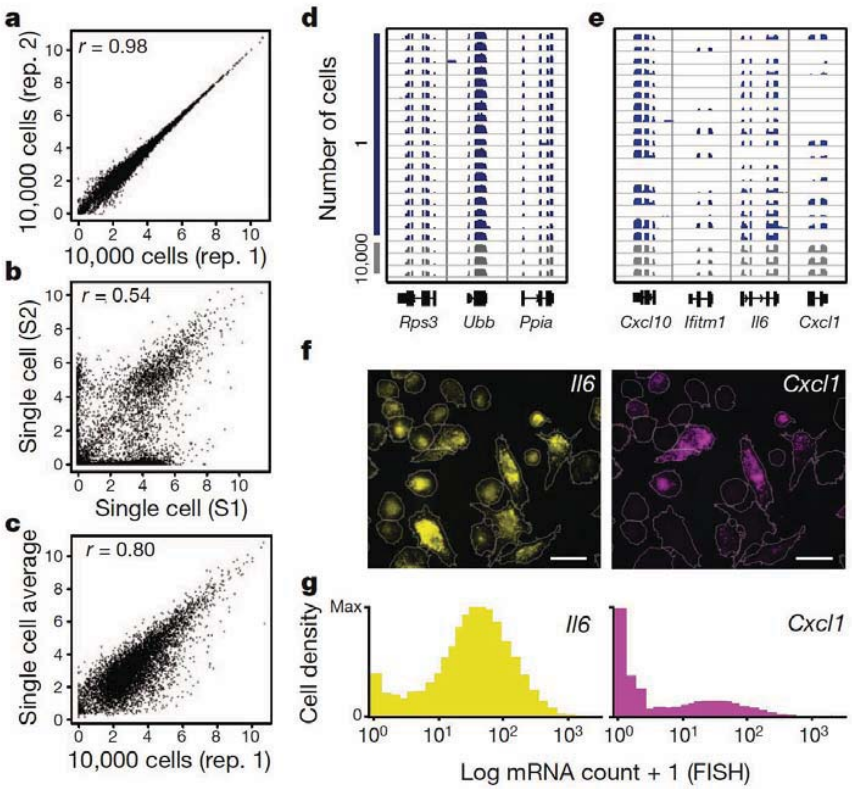
## 2. Why single cell? Bulk RNA-seq cannot be used for cellular heterogeneity





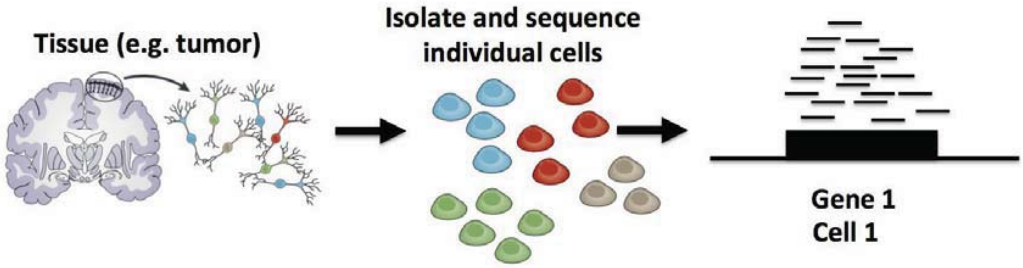

## 2. Why single cell? scRNA-seq uncover bulk cell expression patterns as well as single-cell-level heterogeneity




Shalek et al. (2013) Nature 498, 236-240

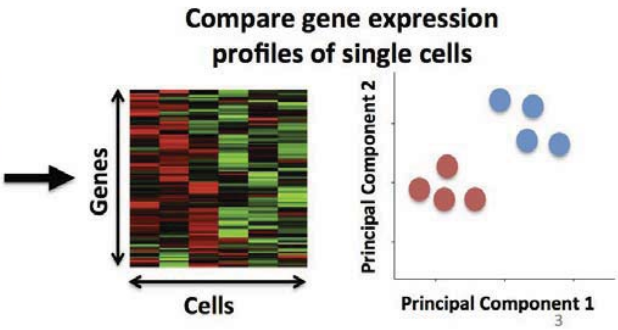
# 3. Single cell technology

## Single-cell RNA-Seq (scRNA-Seq)




**Read Counts**

| | Cell 1 | Cell 2 | ... |
|---|---|---|---|
| Gene 1 | 18 | 0 | |
| Gene 2 | 1010 | 506 | |
| Gene 3 | 0 | 49 | |
| Gene 4 | 22 | 0 | |
| ... | | | |

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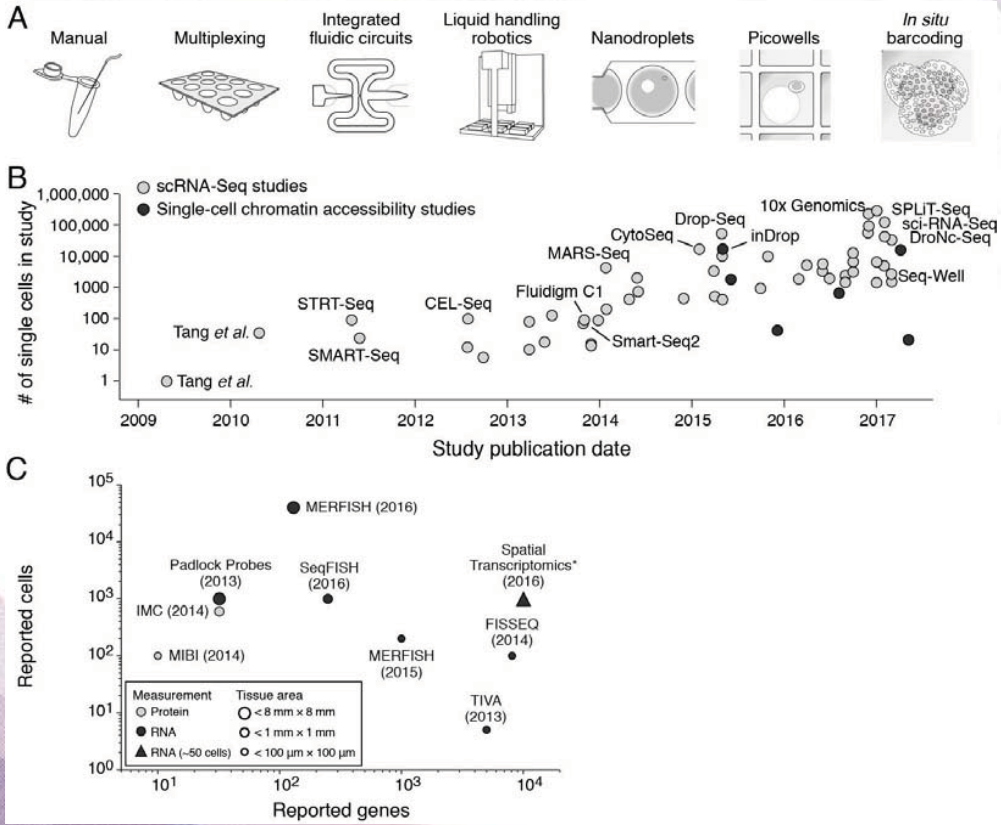
# 3. Single cell technology

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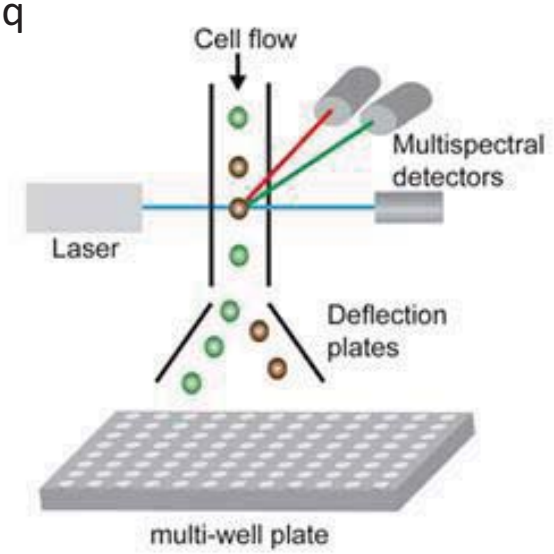
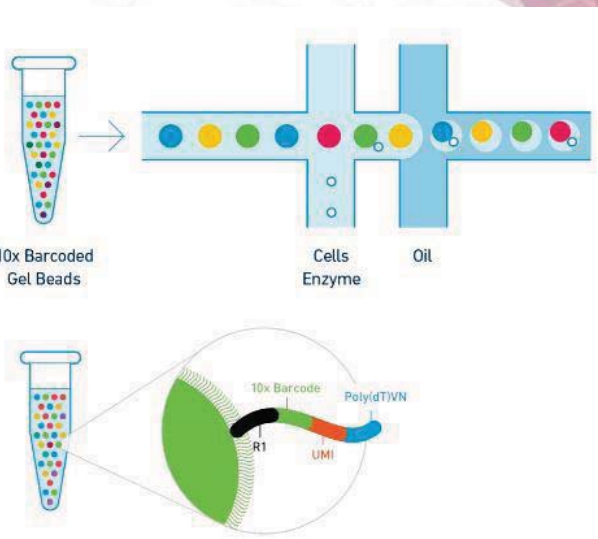
# 3. Single cell technology

Smart-seq: Well-based scRNA-seq




10x Genomics: Microfluidic droplet-based scRNA-seq






# 3. Single cell technology: First single cell RNA-seq paper (2009)

## mRNA-Seq whole-transcriptome analysis of a single cell

Fuchou Tang[1,3], Catalin Barbacioru[2,3], Yangzhou Wang[2], Ellen Nordman[2], Clarence Lee[2], Nanlan Xu[2], Xiaohui Wang[2], John Bodeau[2], Brian B Tuch[2], Asim Siddiqui[2], Kaiqin Lao[2] & M Azim Surani[1]

**Next-generation sequencing technology is a powerful tool for transcriptome analysis. However, under certain conditions, only a small amount of material is available, which requires more sensitive techniques that can preferably be used at the single-cell level. Here we describe a single-cell digital gene expression profiling assay. Using our mRNA-Seq assay with only a single mouse blastomere, we detected the expression of 75% (5,270) more genes than microarray techniques and identified 1,753 previously unknown splice junctions called by at least 5 reads. Moreover, 8–19% of the genes with multiple known transcript isoforms expressed at least two isoforms in the same blastomere or oocyte, which unambiguously demonstrated the complexity of the transcript variants at whole-genome scale in individual cells. Finally, for *Dicer1*$^{-/-}$ and *Ago2*$^{-/-}$ (*Eif2c2*$^{-/-}$) oocytes, we found that 1,696 and 1,553 genes, respectively, were abnormally upregulated compared to wild-type controls, with 619 genes in common.**

Fuchou Tang

Azim Surani

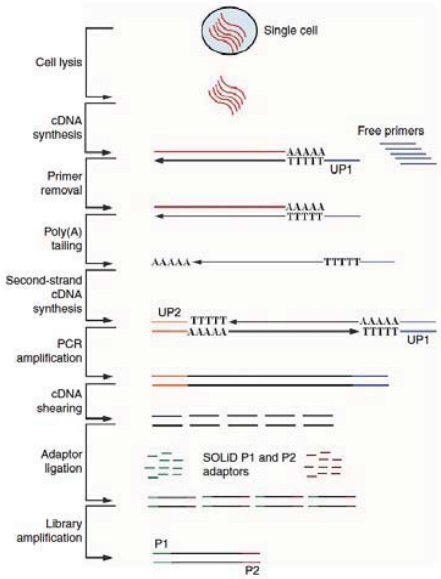
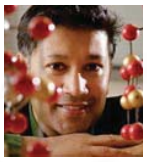



**Figure 1** | Schematic of the single-cell whole-transcriptome analysis. A single cell is manually picked under a microscope and lysed. Then mRNAs are reverse-transcribed into cDNAs using a poly(T) primer with anchor sequence (UP1) and unused primers are digested. Poly(A) tails are added to the first-strand cDNAs at the 3′ end, and second-strand cDNAs are synthesized using poly(T) primers with another anchor sequence (UP2). Then cDNAs are evenly amplified by PCR using UP1 and UP2 primers, fragmented, and P1 and P2 adaptors are ligated to the ends. Finally, emulsion PCR is performed by mixing libraries with 1 μm diameter beads with P1 primers covalently attached to their surfaces.

Question: How to capture the mRNA molecule in a small amount of material?

# 3. Single cell technology: STRT (single cell tagged reverse transcription) – Multiplexing (2011)



Saifu Islam

Sten Linnarsson

Method

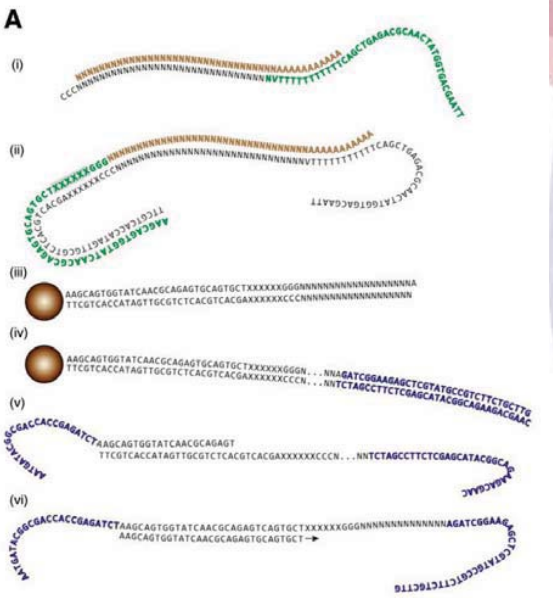
## Characterization of the single-cell transcriptional landscape by highly multiplex RNA-seq

Saiful Islam,[1,4] Una Kjällquist,[1,4] Annalena Moliner,[2] Pawel Zajac,[1] Jian-Bing Fan,[3] Peter Lönnerberg,[1] and Sten Linnarsson[1,5]

[1]Laboratory for Molecular Neurobiology, Department of Medical Biochemistry and Biophysics, Karolinska Institutet, SE-171 77 Stockholm, Sweden; [2]Department of Neuroscience, Karolinska Institutet, SE-171 77 Stockholm, Sweden; [3]Illumina Inc., San Diego, California 92121, USA

Our understanding of the development and maintenance of tissues has been greatly aided by large-scale gene expression analysis. However, tissues are invariably complex, and expression analysis of a tissue confounds the true expression patterns of its constituent cell types. Here we describe a novel strategy to access such complex samples. Single-cell RNA-seq expression profiles were generated, and clustered to form a two-dimensional cell map onto which expression data were projected. The resulting cell map integrates three levels of organization: the whole population of cells, the functionally distinct subpopulations it contains, and the single cells themselves—all without need for known markers to classify cell types. The feasibility of the strategy was demonstrated by analyzing the transcriptomes of 85 single cells of two distinct types. We believe this strategy will enable the unbiased discovery and analysis of naturally occurring cell types during development, adult physiology, and disease.

**Figure 1.** Single-cell tagged reverse transcription (STRT). (*A*) Overview of the method, illustrating the main steps in sample preparation: (*i*) mRNA (brown) is reverse transcribed using a tailed oligo-dT primer (green), generating a first-strand cDNA with 3-6 added cytosines; (*ii*) a helper oligo (green) causes template-switching and thereby introduces a barcode (shaded) and a primer sequence into the cDNA; (*iii*) the product is amplified by single-primer PCR exploiting the template-suppression effect and is then immobilized on beads, fragmented, and A-tailed; (*iv*) the Illumina P2 adapter (blue) is ligated to the free end; (*v*) the P1 adapter is introduced in the library PCR step, using a primer tailed with the P1 sequence (blue); and (*vi*) the final library is sequenced from the P1 side using a custom primer. Each read (arrow) begins by the barcode, followed by three to six Cs, followed by the mRNA insert. (*B*) Illustration of read mapping and annotation, for a two-exon gene. Reads mapping to the sense strand of exons, as well as to splice junctions, were counted toward the expression of the gene. Reads mapping upstream of, downstream from, or in introns were counted for quality control purposes, and antisense hits were used to judge the background level.




Multiplexing using barcode reduces cell-to-cell amplification bias since single-cell cDNA was pooled before amplification

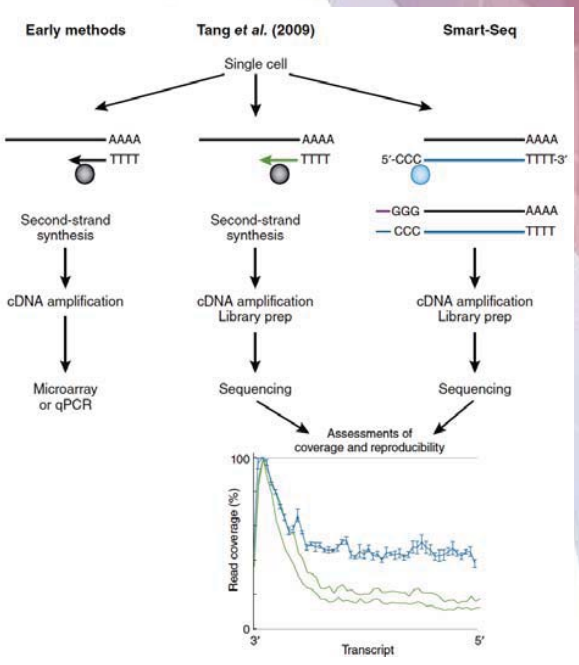


# 3. Single cell technology: SMART(switching mechanism at the 5' end of RNA templates) (2012)

## Full-length mRNA-Seq from single-cell levels of RNA and individual circulating tumor cells

Daniel Ramsköld[1,2,7], Shujun Luo[3,7], Yu-Chieh Wang[4], Robin Li[3], Qiaolin Deng[1], Omid R Faridani[1], Gregory A Daniels[5], Irina Khrebtukova[3], Jeanne F Loring[4], Louise C Laurent[6], Gary P Schroth[3] & Rickard Sandberg[1,2]

Genome-wide transcriptome analyses are routinely used to monitor tissue-, disease- and cell type–specific gene expression, but it has been technically challenging to generate expression profiles from single cells. Here we describe a robust mRNA-Seq protocol (Smart-Seq) that is applicable down to single cell levels. Compared with existing methods, Smart-Seq has improved read coverage across transcripts, which enhances detailed analyses of alternative transcript isoforms and identification of single-nucleotide polymorphisms. We determined the sensitivity and quantitative accuracy of Smart-Seq for single-cell transcriptomics by evaluating it on total RNA dilution series. We found that although gene expression estimates from single cells have increased noise, hundreds of differentially expressed genes could be identified using few cells per cell type. Applying Smart-Seq to circulating tumor cells from melanomas, we identified distinct gene expression patterns, including candidate biomarkers for melanoma circulating tumor cells. Our protocol will be useful for addressing fundamental biological problems requiring genome-wide transcriptome profiling in rare cells.




SMART-seq focus on the full-length transcript coverage. SMART-seq can achieve the coverage by using a reverse transcriptase enzyme from the Moloney murine leukemia virus (template switching and terminal transferase activity).

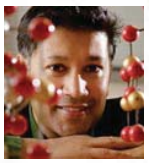
Daniel Ramsköld

Rickard Sandberg

# 3. Single cell technology: UMI (2013)

## Quantitative single-cell RNA-seq with unique molecular identifiers

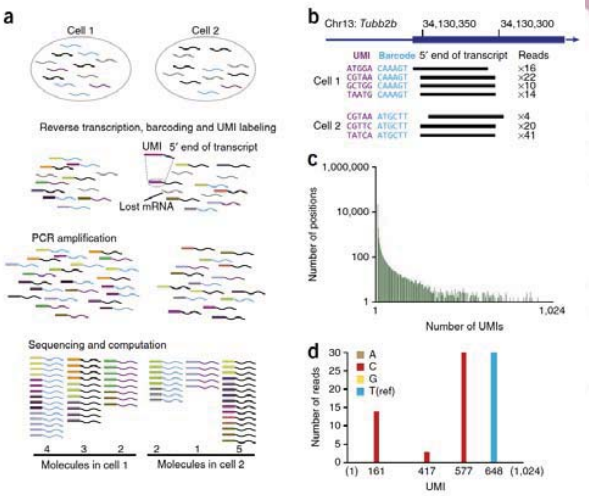
Saiful Islam[1], Amit Zeisel[1], Simon Joost[2], Gioele La Manno[1], Pawel Zajac[1], Maria Kasper[2], Peter Lönnerberg[1] & Sten Linnarsson[1]



Saifu Islam

Sten Linnarsson

**Single-cell RNA sequencing (RNA-seq) is a powerful tool to reveal cellular heterogeneity, discover new cell types and characterize tumor microevolution. However, losses in cDNA synthesis and bias in cDNA amplification lead to severe quantitative errors. We show that molecular labels—random sequences that label individual molecules—can nearly eliminate amplification noise, and that microfluidic sample preparation and optimized reagents produce a fivefold improvement in mRNA capture efficiency.**




UMI enables to quantify the mRNA eliminating amplification noise.

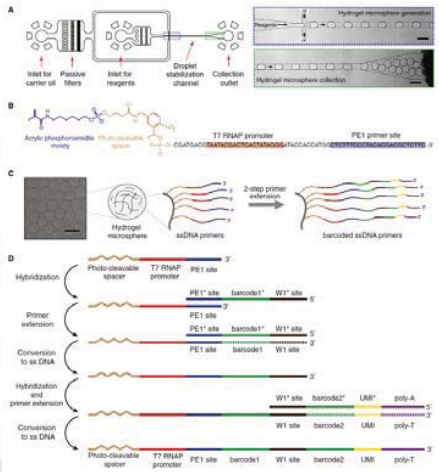


# 3. Single cell technology: inDrops, cell (2015), page 1187-1201 / Drop-seq, Cell (2015), page 1202-1214

## Droplet Barcoding for Single-Cell Transcriptomics Applied to Embryonic Stem Cells

Allon M. Klein,[1,6] Linas Mazutis,[2,3,6] Ilke Akartuna,[2,6] Naren Tallapragada,[1] Adrian Veres,[1,4,5] Victor Li,[1] Leonid Peshkin,[1] David A. Weitz,[2,*] and Marc W. Kirschner[1,*]
[1]Department of Systems Biology, Harvard Medical School, Boston, MA 02115, USA
[2]School of Engineering and Applied Sciences (SEAS), Harvard University, Cambridge, MA 02138, USA
[3]Vilnius University Institute of Biotechnology, Vilnius LT-02241, Lithuania
[4]Department of Stem Cell and Regenerative Biology, Harvard University, Cambridge, MA 02138, USA
[5]Harvard Stem Cell Institute, Harvard University, Cambridge, MA 02138, USA
[6]Co-first author
*Correspondence: weitz@seas.harvard.edu (D.A.W.), marc@hms.harvard.edu (M.W.K.)
http://dx.doi.org/10.1016/j.cell.2015.04.044

Allon Klein

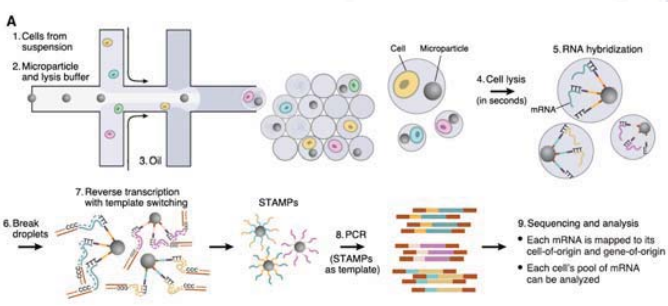



inDrops enables to measure transcriptome of tens of thousands of cells by using microfluidic droplets suspended in carrier oil and barcoding system with UMI.

## Highly Parallel Genome-wide Expression Profiling of Individual Cells Using Nanoliter Droplets

Evan Z. Macosko,[1,2,3,*] Anindita Basu,[4,5] Rahul Satija,[4,6,7] James Nemesh,[1,2,3] Karthik Shekhar,[1] Melissa Goldman,[1,2] Itay Tirosh,[4] Allison R. Bialas,[8] Nolan Kamitaki,[1,2,3] Emily M. Martersteck,[9] John J. Trombetta,[4] David A. Weitz,[8,10] Joshua R. Sanes,[9] Alex K. Shalek,[4,11,12] Aviv Regev,[4,13,14] and Steven A. McCarroll[1,2,3,*]
[1]Department of Genetics, Harvard Medical School, Boston, MA 02115, USA
[2]Stanley Center for Psychiatric Research, Broad Institute of Harvard and MIT, Cambridge, MA 02142, USA
[3]Program in Medical and Population Genetics, Broad Institute of Harvard and MIT, Cambridge, MA 02142, USA
[4]Klarman Cell Observatory, Broad Institute of Harvard and MIT, Cambridge, MA 02142, USA
[5]School of Engineering and Applied Sciences, Harvard University, Cambridge, MA 02138, USA
[6]New York Genome Center, New York, NY 10013, USA
[7]Department of Biology, New York University, New York, NY 10003, USA
[8]The Program in Cellular and Molecular Medicine, Children's Hospital Boston, Boston, MA 02115, USA
[9]Department of Molecular and Cellular Biology and Center for Brain Science, Harvard University, Cambridge, MA 02138, USA
[10]Department of Physics, Harvard University, Cambridge, MA 02138, USA
[11]Ragon Institute of MGH, MIT, and Harvard, Cambridge, MA 02139, USA
[12]Institute for Medical Engineering and Science and Department of Chemistry, MIT, Cambridge, MA 02139, USA
[13]Department of Biology, MIT, Cambridge, MA 02139, USA
[14]Howard Hughes Medical Institute, Chevy Chase, MD 20815, USA
*Correspondence: emacosko@genetics.med.harvard.edu (E.Z.M.), mccarroll@genetics.med.harvard.edu (S.A.M.)
http://dx.doi.org/10.1016/j.cell.2015.05.002

Evan Macosko




Drop-seq enables to measure transcriptome of tens of thousands of cells by using aqueous droplets and barcoding system with UMI.

# 3. Single cell technology (history)

Fuchou Tang

**2009: First single cell RNA-seq paper**

- PolyT primer
- PCR

Sten Linnarsson

**2011: STRT (single cell tagged reverse transcription) - Multiplexing**

- PolyT primer
- PCR
- Cell Barcode
- Template switching

Rickard Sandberg

**2012: SMART(switch mechamism at the 5' end of RNA templates)-seq**

- PolyT primer
- PCR
- Template switching (CCC)

Itai Yanai

**2012: CEL-seq**

- PolyT primer
- IVT
- Cell Barcode

**2013: Smart-seq2**

- PolyT primer
- PCR
- Template switching (LNA)

Rickard Sandberg

**2013: UMI**

- PolyT primer
- PCR
- Cell Barcode
- Template switching
- UMI

Sten Linnarsson

**2014: MARS(Massively parallel RNA single-cell)-seq**

- PolyT primer
- IVT
- Cell Barcode
- FACS
- UMI

Ido Amit

**2015: inDrops, Drop-seq**

- PolyT primer
- IVT (inDrops) PCR (Drop-seq)
- Cell Barcode
- Droplets
- UMI

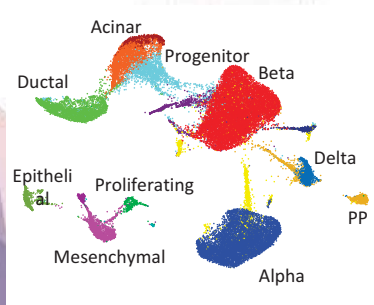
Allon Klein

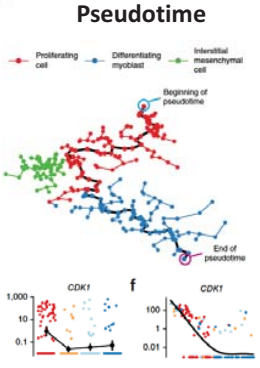
Evan Macosko



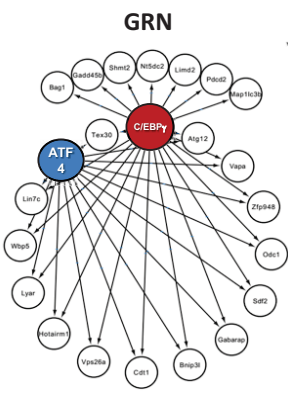
# 4. What can we do using single cell transcriptomics?

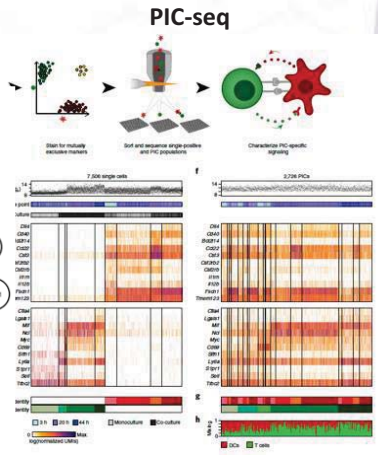
- Heterogeneity → Cell type identification
- Dynamics → Cell type differentiation trajectory / Gene regulatory network reconstruction → Identifying key molecules regulating cellular behavior
- Interactions → Cellular communication



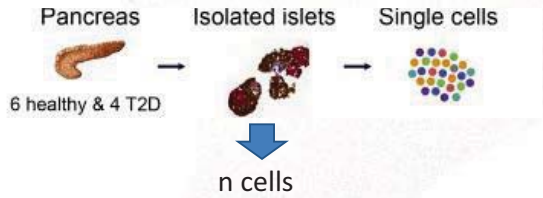

Trapnell et al., Nature Biotech. (2014)



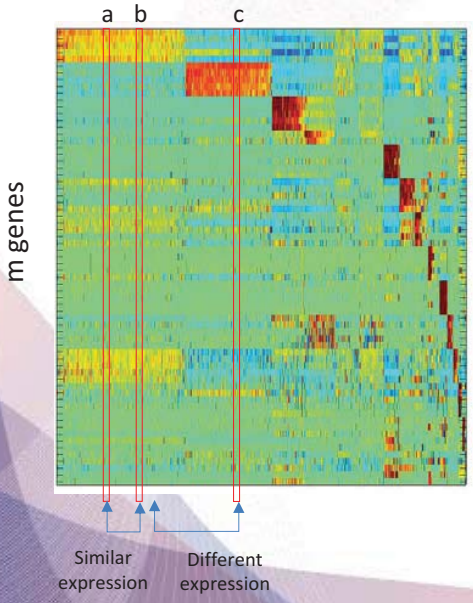




Giladi et al., Nature Biotech. (2020)

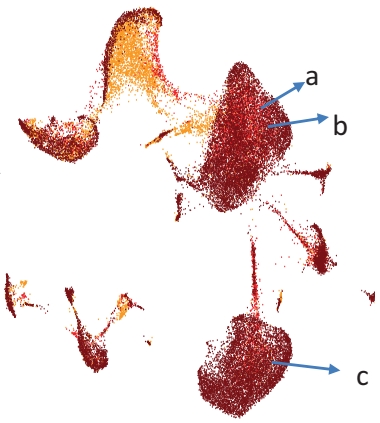
# 4. What can we do using single cell transcriptomics? – Dimension reduction and clustering



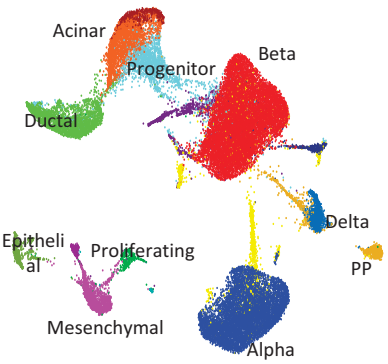

n cells

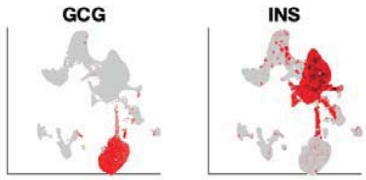



Dimension reduction (PCA, tSNE, UMAP)



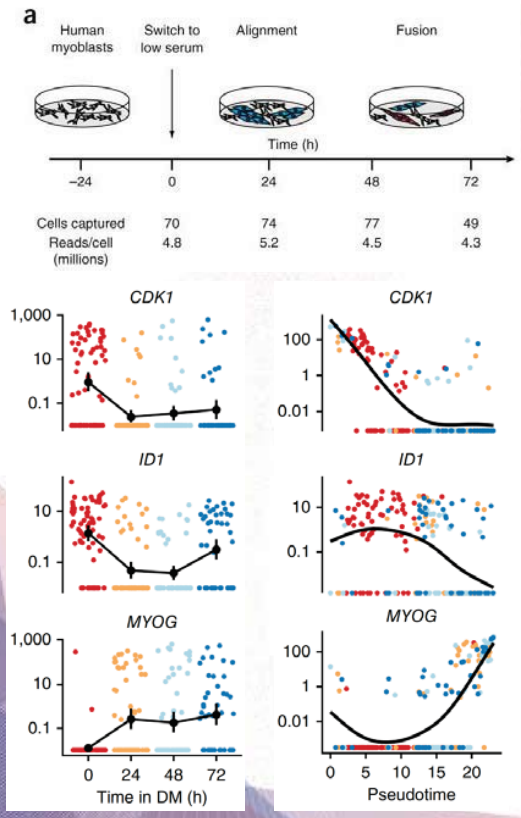

Clustering and cell type annotations

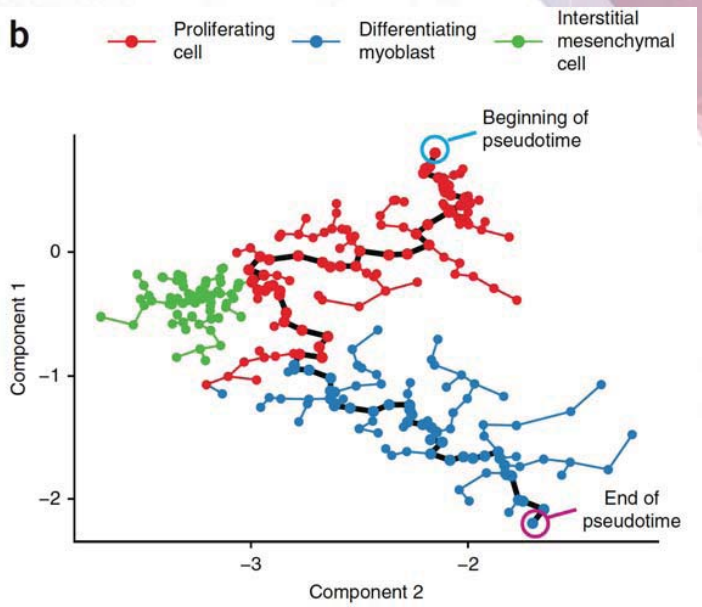





# 4. What can we do using single cell transcriptomics? – Dynamics: Pseudotime analysis

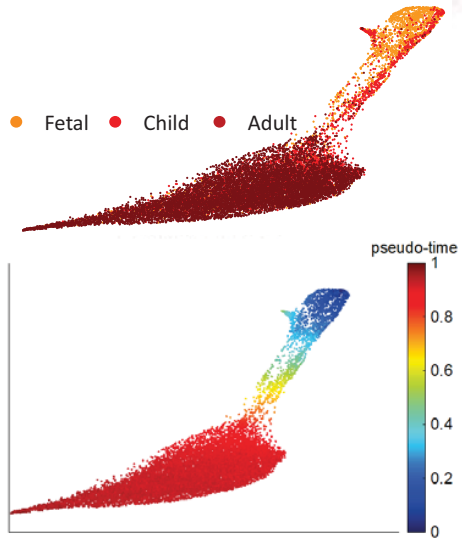


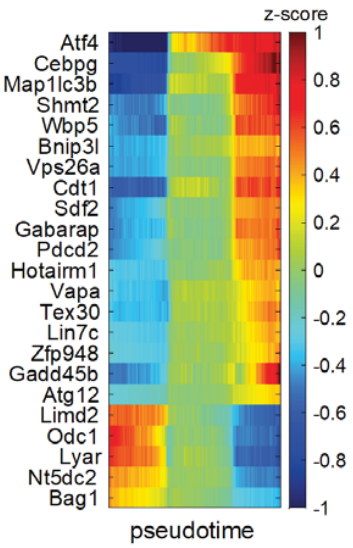

Trapnell et al., Nature Biotech. (2014)

# 4. What can we do using single cell transcriptomics? – Dynamics: Pseudotime analysis

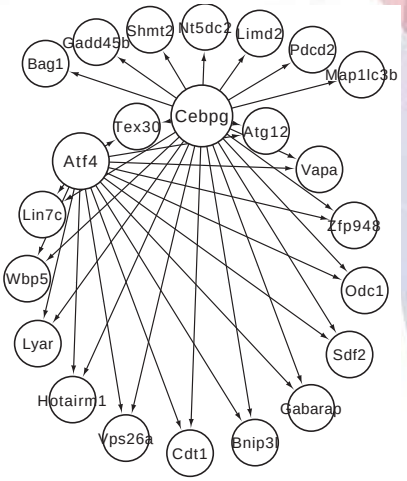
Pseudotime analysis




Gene-gene causal relationships



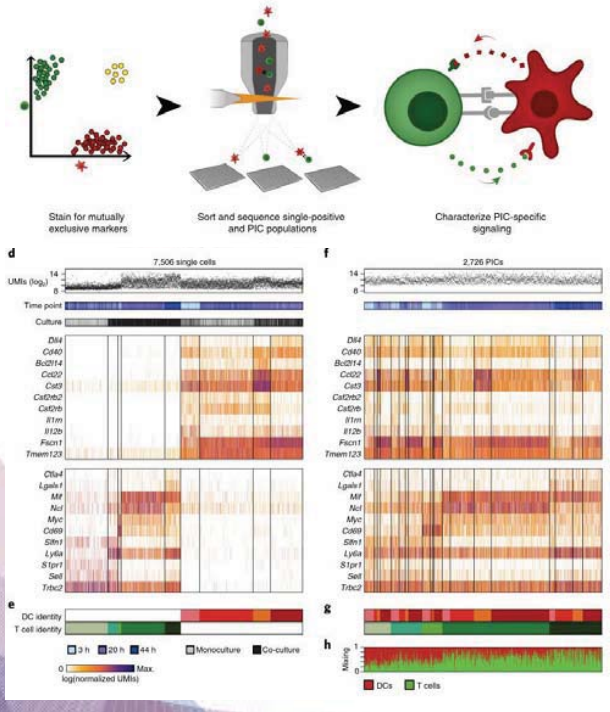

Gene regulatory networks





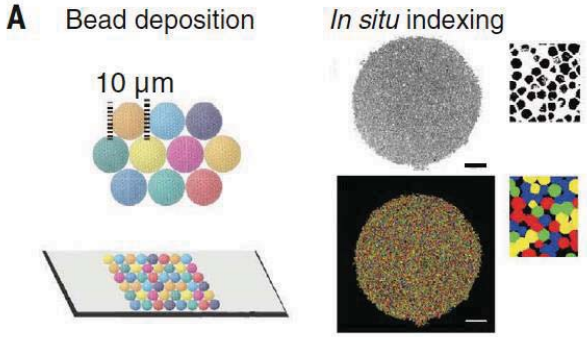

# 4. What can we do using single cell transcriptomics? – Beyond single cell

PIC-seq (two-cells)



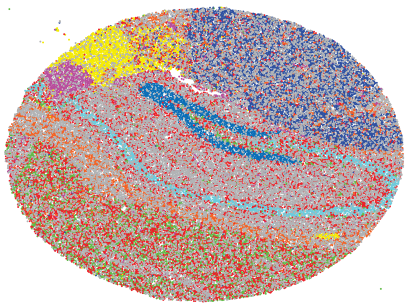

Giladi et al., Nature Biotech. (2020)

Spatial transcriptomics

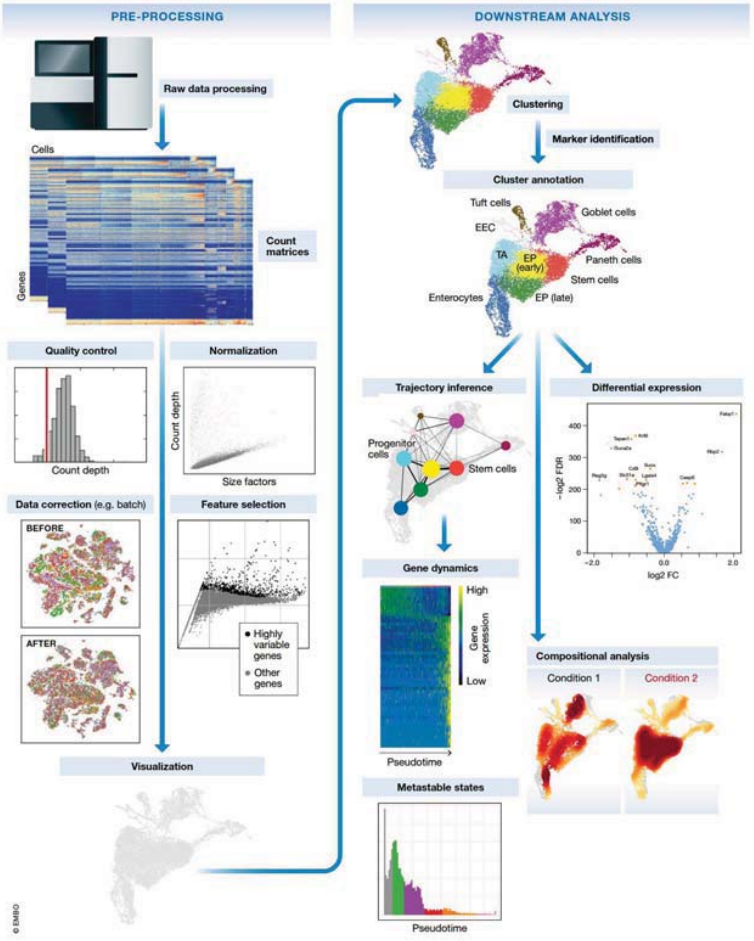



Rodriques et al., Science (2019)

Mouse hippocampus data



## 5. Single cell transcriptomics data analysis pipeline

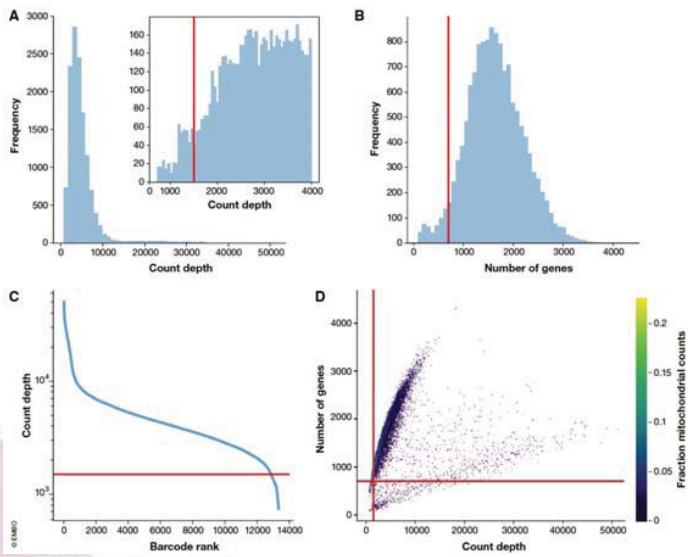





## 5. Single cell transcriptomics data analysis pipeline: quality control

- For cell QC, we must filter out dying cells or doublets.
- Three QC criterion
  1. The **number of counts** per barcode
  2. The **number of genes** per barcode
  3. The fraction of counts from **mitochondrial gene** per barcode
- Example 1: cells with low counts, few detected genes, and a high fraction of mitochondrial genes may represent dying cells
- Example 2: cells with high counts and a large number of detected genes may represent doublets

# 5. Single cell transcriptomics data analysis pipeline: quality control

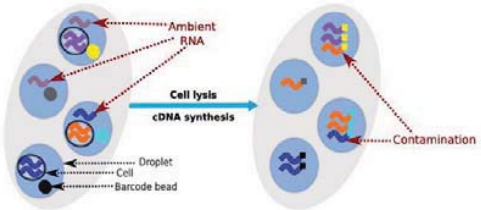



- Considering only one criteria can lead to misinterpretation of cellular signals
  - High fraction of mitochondrial counts may be involved in respiratory processes
  - Low counts and genes may correspond to quiescent cell populations
  - High counts and genes may correspond large cell size (adipocytes)



# 5. Single cell transcriptomics data analysis pipeline: quality control

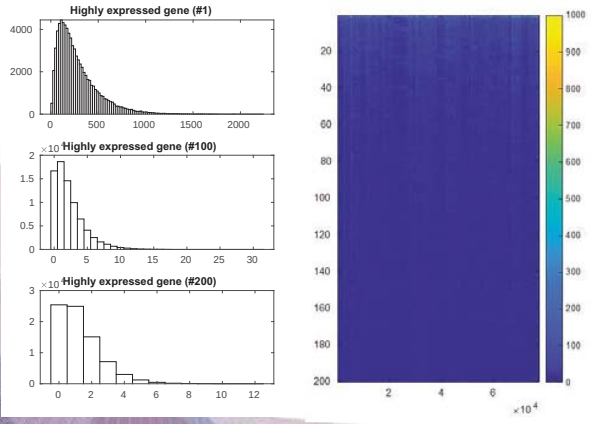
- For **gene QC**, we can filter out genes that are not expressed in more than a few cells
  - You need to consider the minimum number of cell cluster size of your interest when you choose the threshold.
  - For example, you need to lower the threshold, when you tried to find a rare cell type.
- **Ambient gene expression** (counts that do not originate from a barcoded cell, but from other lysed cells whose mRNA contaminated the cell suspension prior to library construction)
  - For example, super-highly expressed genes can be detected in an empty droplet. SoupX and DecontX provide a correction for this contaminzation in droplet-based scRNA-seq datasets



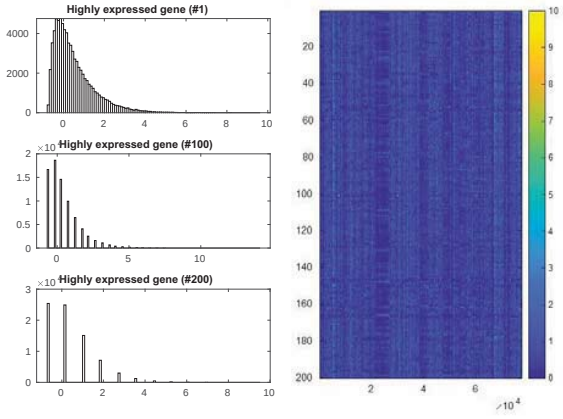
## 5. Single cell transcriptomics data analysis pipeline: normalization

- **Gene normalization / scaling (z scores)**: scaling gene counts to have zero mean and unit variance. There is no consensus on whether or not to do this.
  - Should all genes be weighted equally? versus
  - Is the magnitude of expression of a gene an informative for the importance of the gene?

Count data




Z scores

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## 5. Single cell transcriptomics data analysis pipeline: normalization

- **Log normalization**: log2(x+1) This is a useful tool for the downstream analysis (DEG, clustering, etc.)! ➔
  1. Distance between log-transformed expression values represent **log fold changes**
  2. Log transformation mitigates the **mean-variance relationship**. Expression variance of a gene can be considered as the importance of the gene. Then, the importance of lowly-expressed genes can be ignored.
  3. Log transformation reduces the **skewness** of the data to approximate the **assumption** of many downstream analysis tools that the data are **normally distributed**

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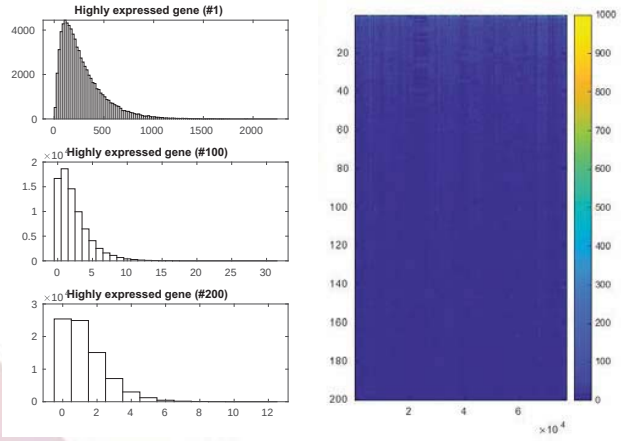
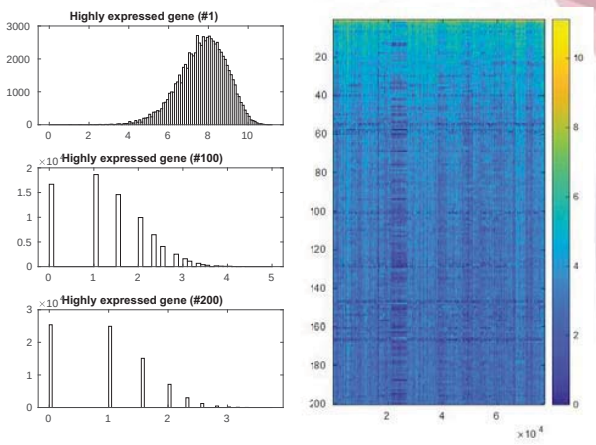
# 5. Single cell transcriptomics data analysis pipeline: normalization

Count data



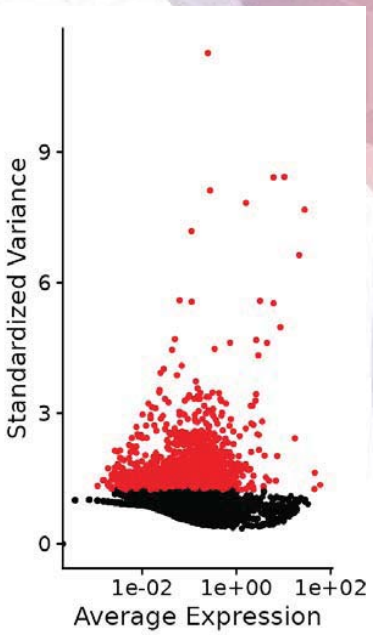

Log transformed






# 5. Single cell transcriptomics data analysis pipeline: feature selection

- Depending on the task and the complexity of the dataset, typically between 1,000 and 5,000 Highly Variable Genes (HVGs) are selected.
- Seurat procedure
  1. Genes are binned by their **mean expression of count** data
  2. The genes with the highest **variance-to-mean ratio** are selected as HVGs



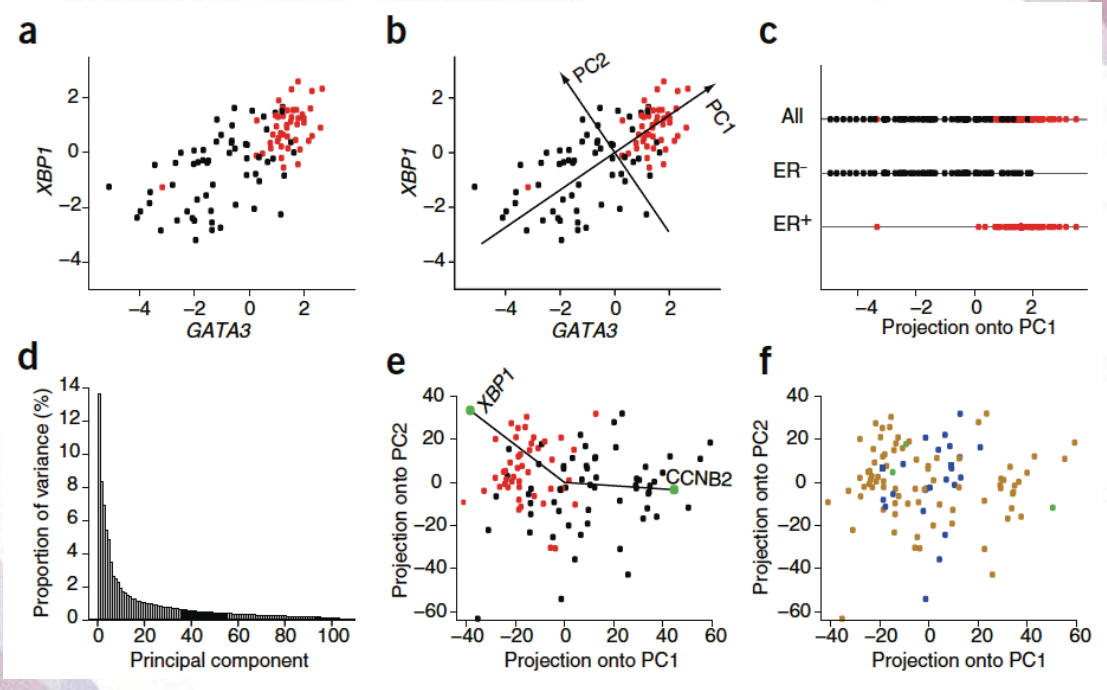
# 5. Single cell transcriptomics data analysis pipeline: dimension reduction and visualization

- Dimensionality reduction algorithms **embed** the expression matrix into a **low-dimensional space**, which is designed to capture the **underlying structure** in the data in as few dimensions as possible.
- Two main objectives:
  1. Visualization: optimally describe the dataset in 2D or 3D
  2. Summarization: can be used to reduce the data to its essential components by finding the inherent dimensionality
- 2D visualization cannot be used for summarization **(Visualization ≠ Summarization)**
- Linear method: PCA
- Non-linear methods: t-SNE, Diffusion maps, UMAP, SPRING's force-directed layout.

*Dimensionality reduction
#Visualization



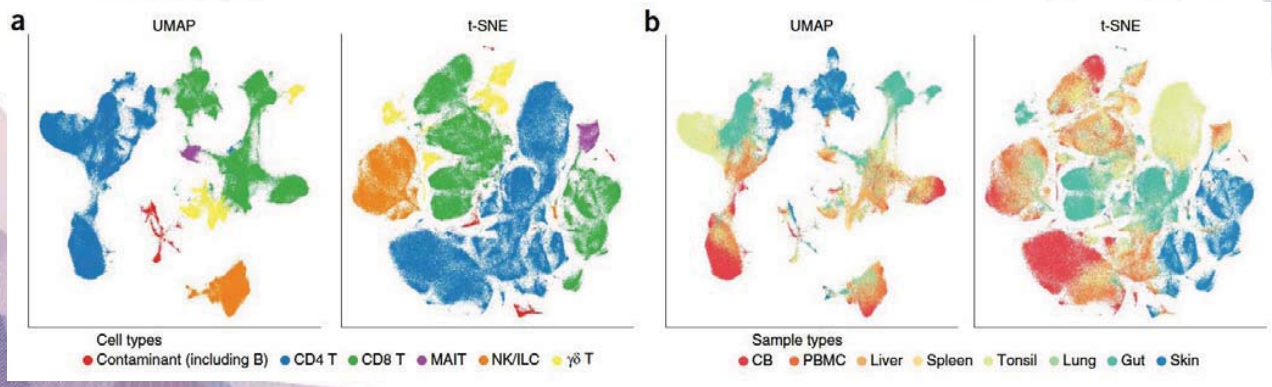
# 5. Single cell transcriptomics data analysis pipeline: dimension reduction and visualization - PCA




Ringner, "What is principal component analysis?", Nature Biotechnology 2008

# 5. Single cell transcriptomics data analysis pipeline: dimension reduction and visualization - PCA

- t-SNE suffers from limitations such as loss of large-scale information (the inter-cluster relationships) and inability to meaningfully represent very large datasets.
- Both UMAP and t-SNE were successfully pulling together only clusters corresponding to similar cell populations.
- However, t-SNE separated cell populations into distinct clusters than UMAP.

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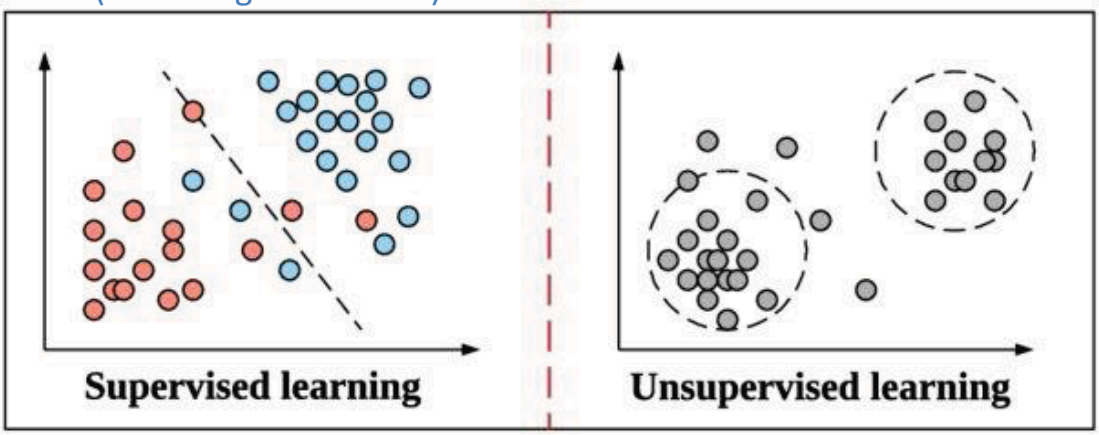
# 5. Single cell transcriptomics data analysis pipeline: clustering

- Clustering allows us to infer the **identity** of member cells.
- Clusters are obtained by grouping cells based on the **similarity** (or **distance**) of their gene expression profiles.
- Clustering is a classical **unsupervised machine learning** problem, based directly on a distance matrix.

Supervised learning
(There **is** ground truth)

Unsupervised learning
(**No** ground truth)

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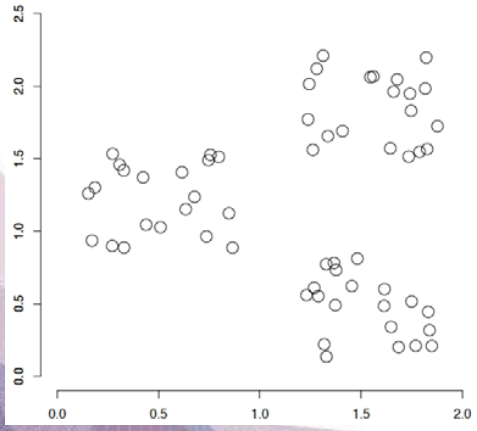
Orchestrating the Development Lifecycle of Machine Learning-Based IoT Applications

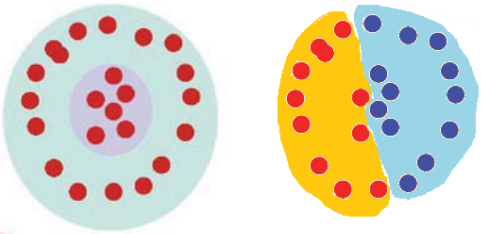
# 5. Single cell transcriptomics data analysis pipeline: clustering

- Two approaches

1. Conventional clustering algorithms: Cells are assigned to clusters by minimizing intracluster distances or finding dense regions in the reduced expression space.
2. Community detection methods: graph-partitioning algorithms and thus rely on a graph representation of single-cell data. (KNN graph)

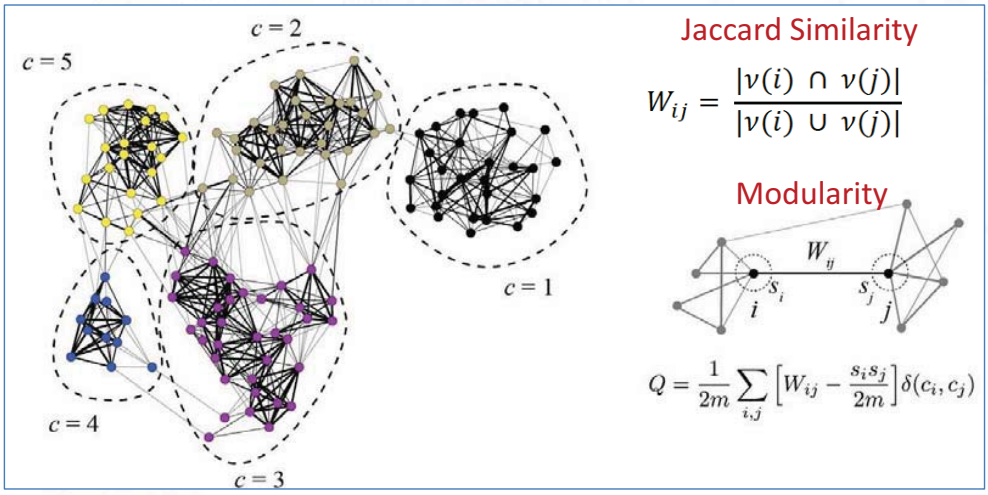



- How would you design an algorithm for finding the three clusters in this case?
- A cluster is a collection of data items which are "similar" between them, and "dissimilar" to data items in other clusters.





# 5. Single cell transcriptomics data analysis pipeline: clustering




Jaccard Similarity

$$W_{ij} = \frac{|\nu(i) \cap \nu(j)|}{|\nu(i) \cup \nu(j)|}$$

Modularity




$$Q = \frac{1}{2m} \sum_{i,j} \left[ W_{ij} - \frac{s_i s_j}{2m} \right] \delta(c_i, c_j)$$

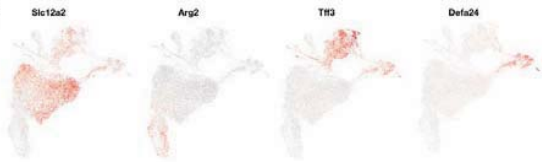
Dana Pe'er

Algorithm

1. Construct a K-nearest neighbor graph using Euclidean distance
2. Construct a weighted shared nearest neighbor graph using Jacarrd similarity
3. Maximize the modularity using the Louvain method

## 5. Single cell transcriptomics data analysis pipeline: cell type identification

- The gene signature a.k.a. ***marker genes*** characterize the cluster and are used to annotate it with a meaningful biological label.
- There are several axes of variation that determine cellular identity
    1. It is **not always clear** what constitutes a cell type. Ex) "T cells" may be a satisfactory label of a cell type to some, others may look for CD4+ and CD8+ T cells.
    2. Cells of the same cell type in **different states** may be detected in separate clusters.
- Identifying and annotating clusters relies on using external sources of information describing the expected expression profiles of individual cell identities.

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## 5. Single cell transcriptomics data analysis pipeline: cell type identification

- Two ways to use reference database information to annotate clusters

1. Data-derived marker genes
    - Differential expression (DE) testing between the cells in one cluster and all other cells in the dataset (typically up-regulated in the cluster of interest) with simple statistical tests (Wilcoxon rank-sum test or the t-test)
    - Null hypothesis: genes have the same distribution of expression values between the two groups.
    - The p-values are often inflated, which can lead overestimation of the number of marker genes. However, the ranking is unaffected. ➔ We can focus on the top-ranked marker genes.
    - Differential gene expression not only depends on the cell cluster but also on the dataset composition.
2. Automated cluster annotation by directly comparing the gene expression profiles of annotated reference clusters to individual cells.

❖**The current best practice is a combination of both approaches.**

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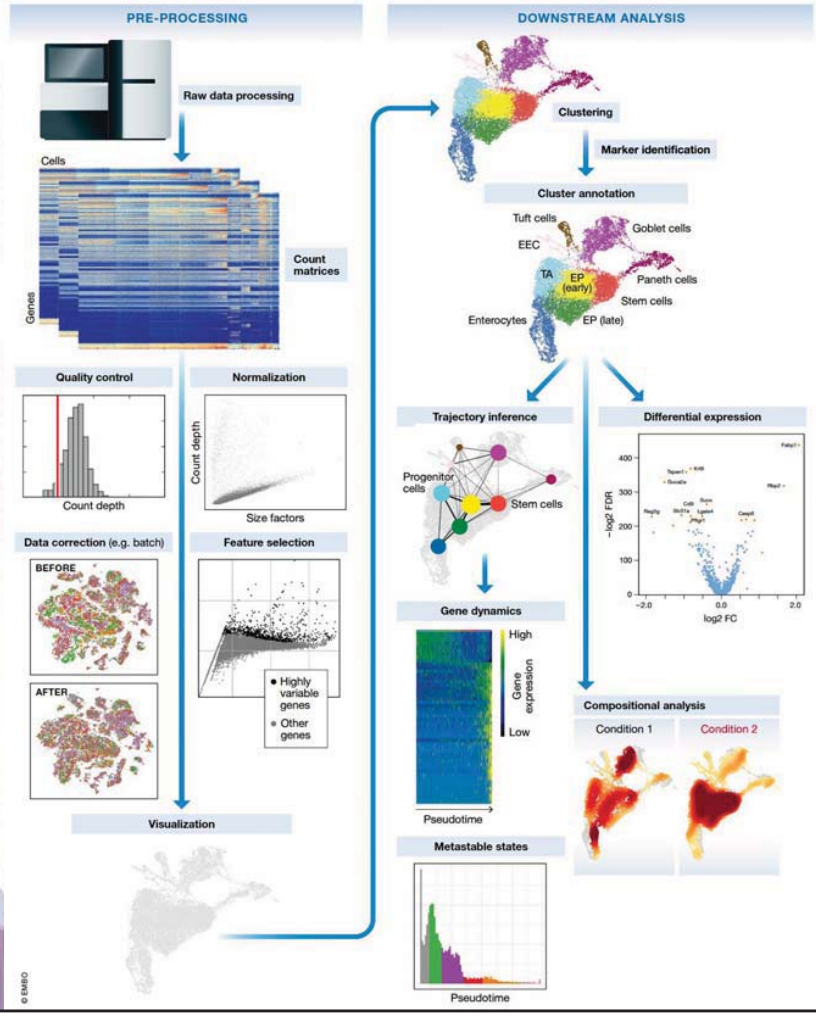
PRE-PROCESSING
Raw data processing
Cells
Genes
Count matrices
Quality control
Count depth
Normalization
Count depth
Size factors
Data correction (e.g. batch)
BEFORE
AFTER
Feature selection
Highly variable genes
Other genes
Visualization
DOWNSTREAM ANALYSIS
Clustering
Marker identification
Cluster annotation
Tuft cells
EEC
Goblet cells
TA
EP (early)
Paneth cells
Stem cells
Enterocytes
EP (late)
Trajectory inference
Progenitor cells
Stem cells
Gene dynamics
High
Gene expression
Low
Pseudotime
Metastable states
Pseudotime
Differential expression
−log2 FDR
log2 FC
Compositional analysis
Condition 1
Condition 2

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