KSBi-BIML 2026

Bioinformatics & Machine Learning(BIML) Workshop for Life Scientists

생명정보학 & 머신러닝 워크샵 (온라인)

Single-cell RNA-sequencing analysis: Assignment of cell types

김규태 _ 아주대학교

KSBI KOREAN SOCIETY FOR BIOINFORMATICS | 한국생명정보학회

⚠

본 강의 자료는 한국생명정보학회가 주관하는 BIML 2026 워크샵을 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 행위자 본인에게 있음**을 알립니다.

KSBi-BIML 2026

Bioinformatics & Machine Learning (BIML) Workshop for Life Scientists

한국생명정보학회가 주최하는 BIML-2026 동계 Bioinformatics &Machine Learning 교육 워크숍에 여러분을 초대합니다.

BIML 워크숍은 생명정보학 연구자들이 최신 AI바이오 분야의 인공지능 기반 분석 기술과 바이오 데이터 분석 기법을 이론과 실습을 통해 체계적으로 배울 수 있는 전문 교육 프로그램입니다. 2015년에 시작된 BIML 워크숍은 올해로 12년 차를 맞이하며, 국내 생명정보학 분야의 최초이자 최고 수준의 교육 프로그램으로 자리 잡았습니다. 이번 워크숍은 크게 인공지능바이오(AI바이오) 분야와 디지털바이오 분야, 두 분야로 구성됩니다.

AI바이오 분야에서는 생명정보 분석에 폭넓게 응용되고 있는 다양한 인공지능 기반 자료 모델링 기법을 다룰 예정입니다. 특히, 인공지능 심층학습을 활용한 단백질 구조 예측, 유전체 분석, 신약 개발에 대한 이론 및 실습 강의가 진행됩니다.

또한 디지털바이오 분야에서는 단일세포오믹스, 공간오믹스, 멀티오믹스, 메타오믹스에 대한 강의도 마련되어 있어, 연구자들의 분석 역량 강화에 실질적인 도움을 줄 것으로 기대됩니다.

또한 2024년부터 추가된 의료정보 자료 분석을 다루는 강의를 올해도 지속해서 운영하고자 합니다. 이는 최근 의료정보 자료 분석에 관한 연구 수요 증가를 반영한 것으로, 관련 연구를 수행하는 의과학자 및 의료정보 연구자들에게 유용한 지침을 제공할 것입니다.

또한, 올해도 생명정보학 기술의 다양화에 발맞춰 온라인 강좌를 대폭 확대했습니다. 올해는 무료 강좌 10개를 포함한 총 40개 이상의 강좌가 개설되며, 연구 주제에 맞는 강좌 추천과 강연료 할인 혜택도 제공합니다.

BIML-2026는 국내 주요 연구 중심 대학의 전임 교수 및 각 분야 최고 전문가들의 강의로 구성되어 있으며, 기초 이론부터 최신 연구 동향까지 아우르는 심도 있는 교육의 장이 될 것으로 확신합니다.

여러분의 많은 관심과 참여를 기대합니다!

2026년 2월

한국생명정보학회장 류 성 호

강의개요

Single-cell RNA-sequencing analysis: Assignment of cell types

본 강의는 단일세포 전사체 데이터 분석의 기본적인 측면을 다룬다. 단일세포 수준으로 분석하는 것이 왜 중요한지에 대한 개론을 제공하며, 데이터 유형의 구조와 형식을 설명하고, 데이터 전처리 과정을 이해할 수 있도록 이론과 함께 실습 강의를 제공한다. 또한, 단일세포 전사체 데이터를 이용한 세포 유형을 결정하는 전반적인 과정을 이해할 수 있다. 이를 통해 학습자들은 단일세포 연구에서 데이터를 처리하고 세포 유형을 파악하는데 필요한 기초적인 지식을 습득하게 된다.

강의는 다음의 내용을 포함한다:

- 단일세포 전사체 데이터 분석의 중요성과 의의를 이해
- 단일세포 전사체 데이터의 구조와 형식에 대해 학습
- 단일세포 전사체 데이터를 활용하여 세포 유형을 할당하는 과정을 이해

* 교육생준비물:

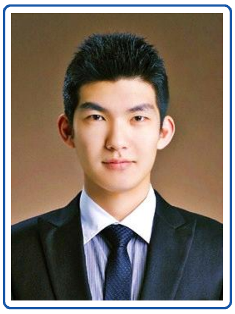
Rstudio 및 Seurat (R package)가 설치된 노트북 (메모리 8GB 이상, 디스크 여유공간 30GB 이상)

* 강의 난이도: 초급

* 강의: 김규태 교수 (아주대학교의과대학 생리학교실)

Curriculum Vitae

Speaker Name: Kyu-Tae Kim, Ph.D.



▶ Personal Info

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Title	Assistant Professor
Affiliation	Ajou University School of Medicine

▶ Contact Information

Address	164, Wolrd cup-ro, Yeongtong-gu, Suwon 16499
Email	kimqtae@ajou.ac.kr

Research Interest

Immunogenomics, Cancer evolution, Computational Biology

Educational Experience

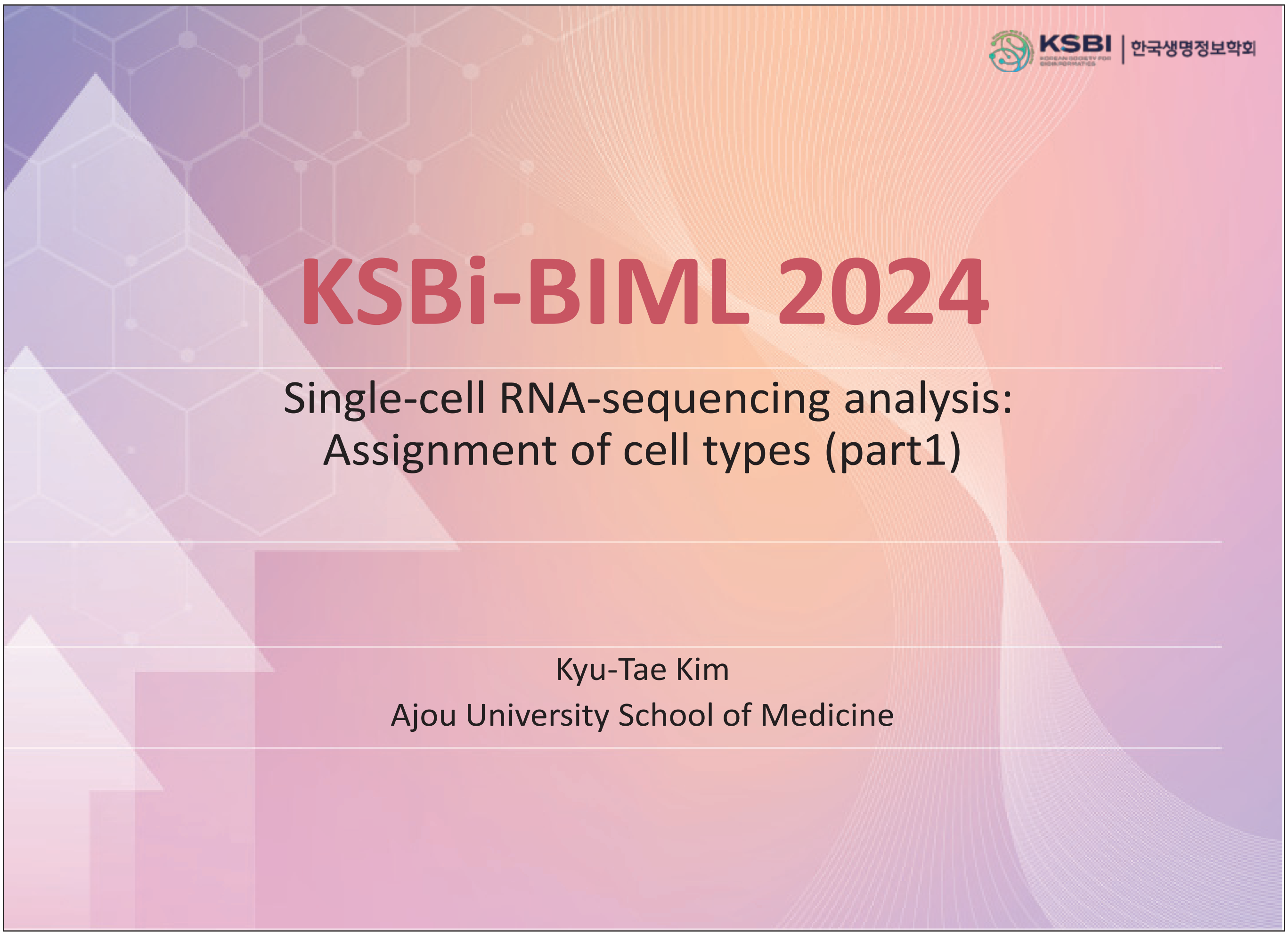
2010	B.S., Konkuk University, Seoul, Korea
2012	M.S., Seoul National University, Seoul, Korea
2015	Ph.D., Seoul National University, Seoul, Korea

Professional Experience

2013-2017	Researcher, Samsung Genome Institute, Samsung Medical Center, Seoul, Korea
2017-2019	Postdoctoral Fellow, New York Genome Center, NY, USA
2020-	Assistant Professor, Ajou University School of Medicine, Suwon, Korea

Selected Publications (5 maximum)

1. Determinants of Response and Intrinsic Resistance to PD-1 Blockade in Microsatellite Instability-High Gastric Cancer, Cancer Discovery, 2021 (corresponding author)
2. Somatic mutations and cell identity linked by Genotyping of Transcriptomes, Nature, 2019 (first author)
3. SIDR: simultaneous isolation and parallel sequencing of genomic DNA and total RNA from single cells, Genome Research, 2018 (first author)
4. Application of single-cell RNA sequencing in optimizing a combinatorial therapeutic strategy in metastatic renal cell carcinoma, Genome Biology, 2016 (first author)
5. Single-cell mRNA sequencing identifies subclonal heterogeneity in anti-cancer drug responses of lung adenocarcinoma cells, Genome Biology, 2015 (first author)



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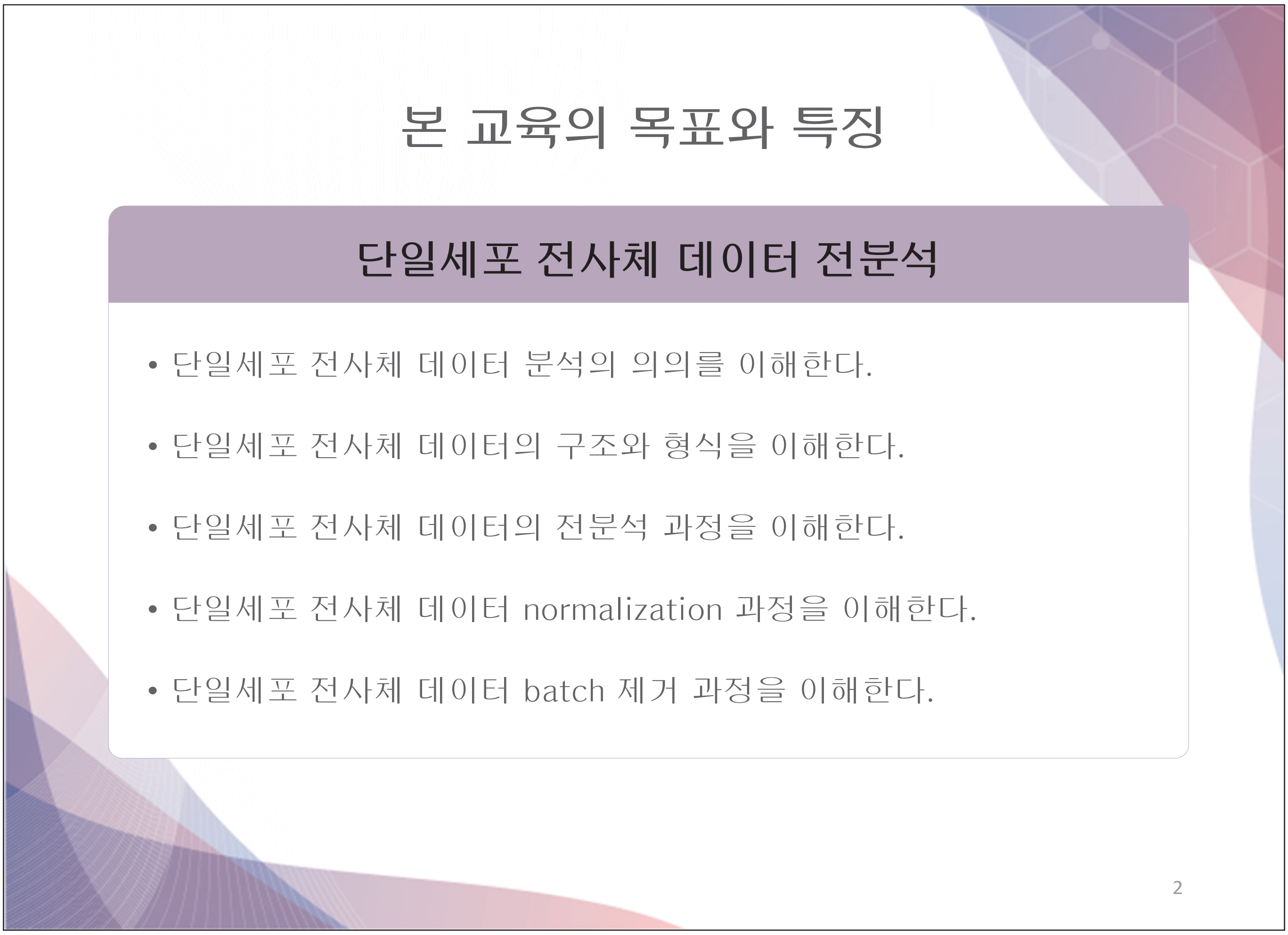
Single-cell RNA-sequencing analysis: Assignment of cell types (part1)

Kyu-Tae Kim
Ajou University School of Medicine

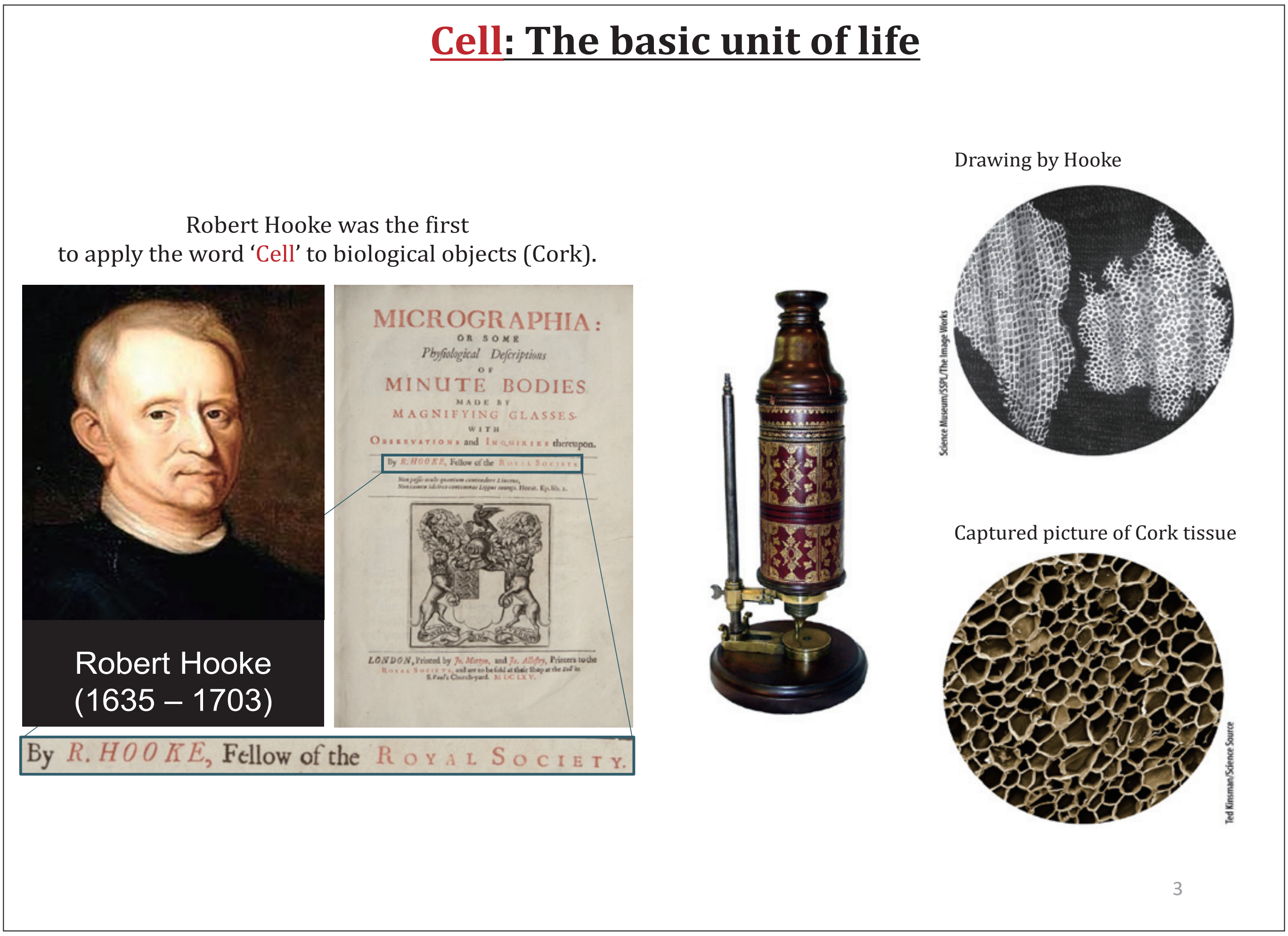


본 교육의 목표와 특징

단일세포 전사체 데이터 전분석

- 단일세포 전사체 데이터 분석의 의의를 이해한다.
- 단일세포 전사체 데이터의 구조와 형식을 이해한다.
- 단일세포 전사체 데이터의 전분석 과정을 이해한다.
- 단일세포 전사체 데이터 normalization 과정을 이해한다.
- 단일세포 전사체 데이터 batch 제거 과정을 이해한다.

2



Cell: The basic unit of life

Robert Hooke was the first
to apply the word ‘Cell’ to biological objects (Cork).

Robert Hooke
(1635 – 1703)

MICROGRAPHIA:
OR SOME
Physiological Descriptions
OF
MINUTE BODIES
MADE BY
MAGNIFYING GLASSES.
WITH
OBSERVATIONS and INQUIRIES thereupon.

By R. HOOKE, Fellow of the ROYAL SOCIETY.

Non possis oculo quantum contendere Linceus,
Non tamen idcirco contemnas Lippus inungi. Horat. Ep. lib. 1.

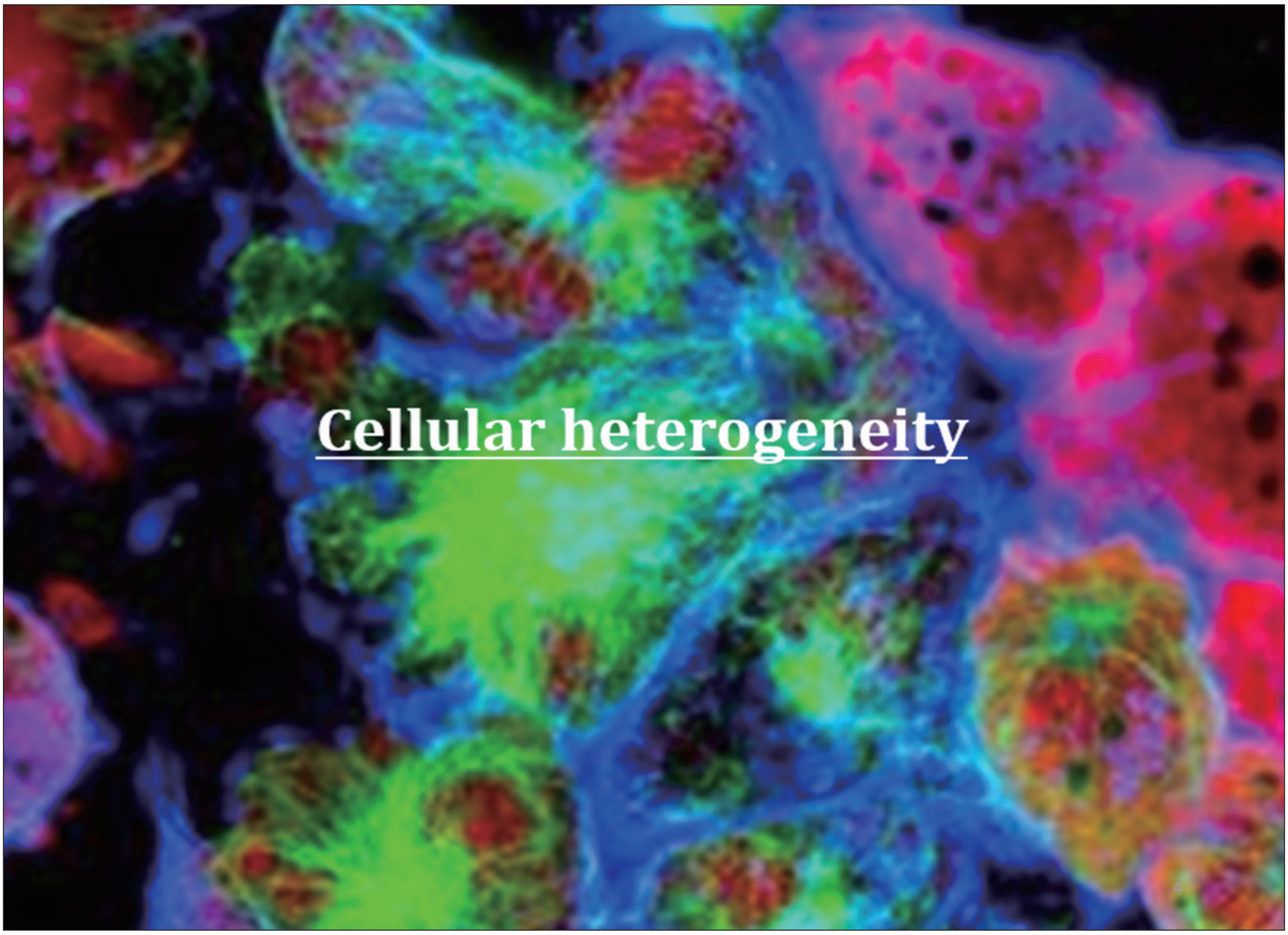
LONDON, Printed by *Jo. Martyn*, and *Ja. Allestry*, Printers to the
ROYAL SOCIETY, and are to be sold at their Shop at the Bell in
S. Paul's Church-yard. MDCLXV.

By R. HOOKE, Fellow of the ROYAL SOCIETY.

Drawing by Hooke

Captured picture of Cork tissue

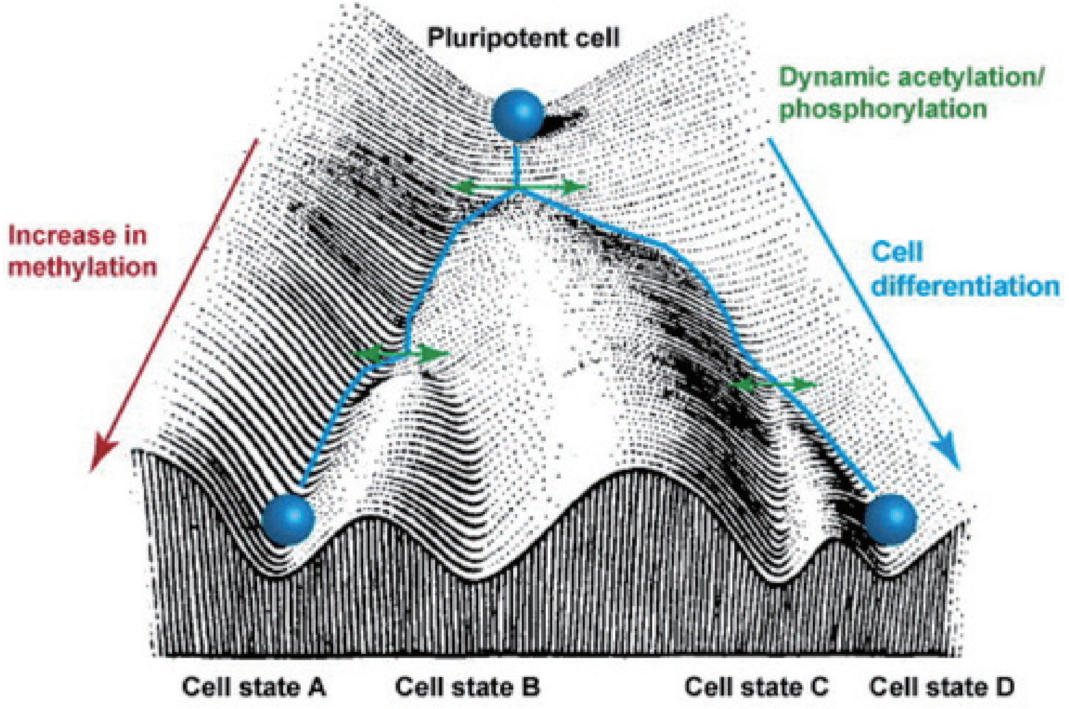
3



Cellular heterogeneity

Cell: The basic unit of life

From a cell, then …

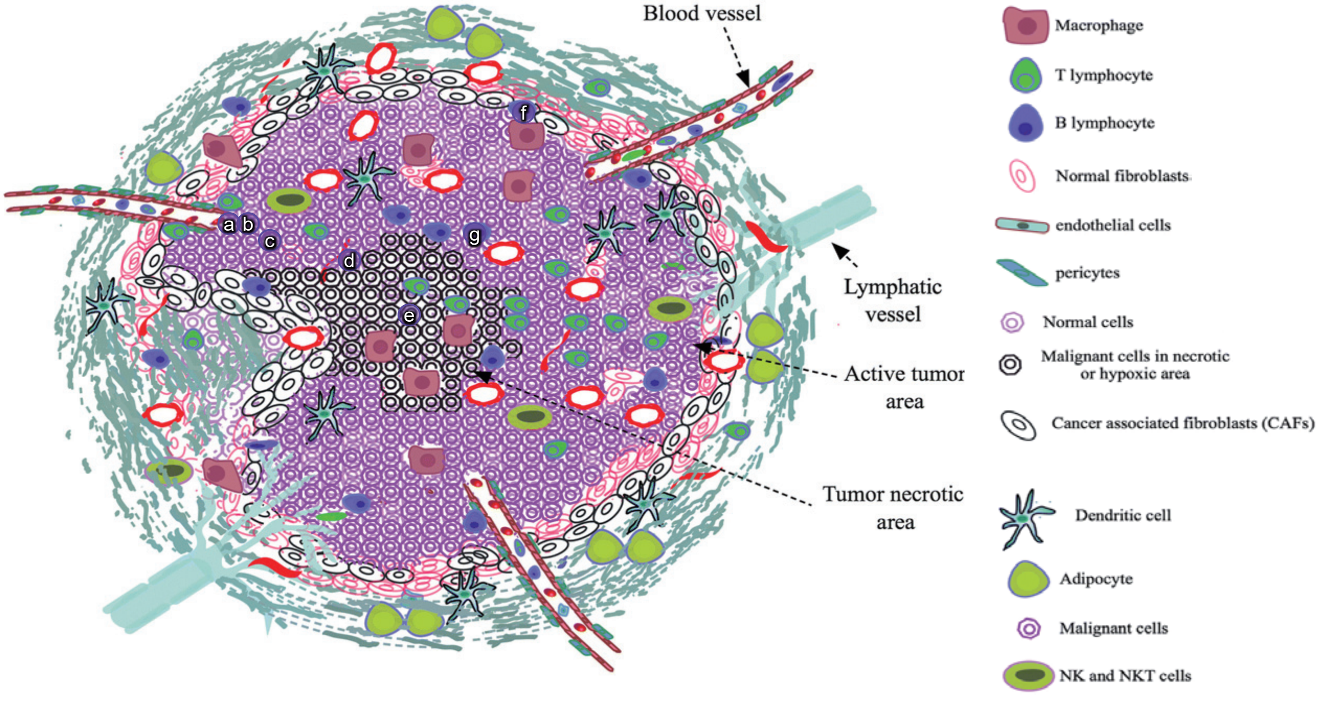


Waddington's model

Cell: The basic unit of life

Tumor Micro-Environment

Within-cell-type differences

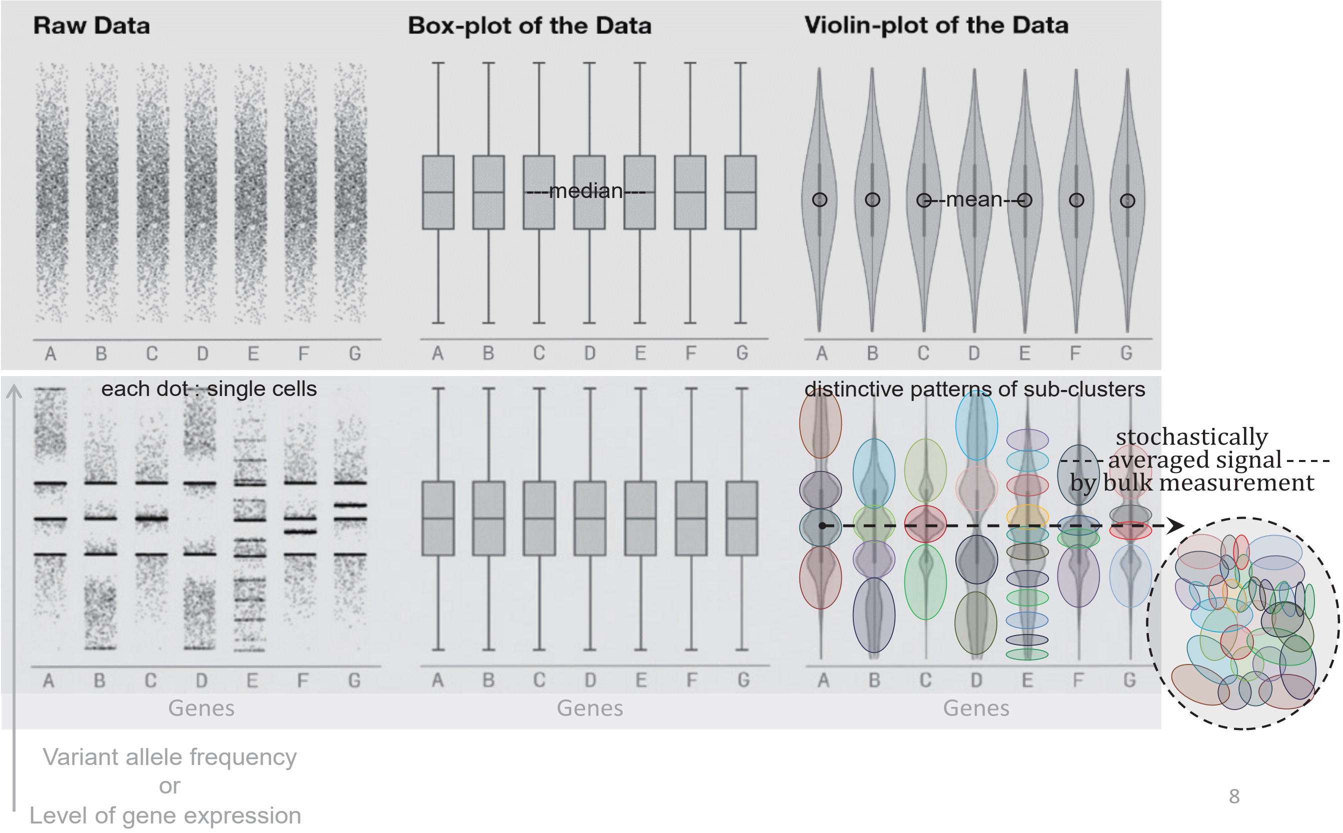


Why single-cell sequencing?

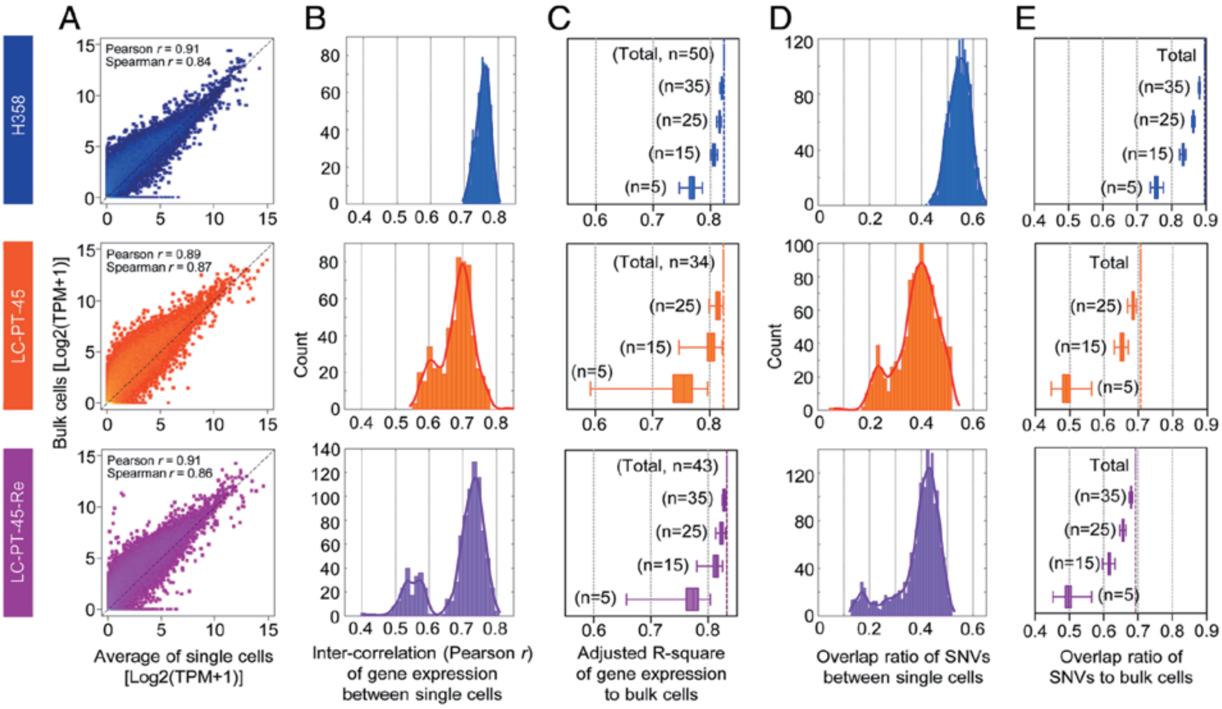
Bulk analysis vs. Single-cell RNA-seq

"No! Sometimes the Sum of the Parts (single-cells) is Greater than the Whole (bulk)."

(original phrase by Aristotle, "The Whole is Greater than the Sum of its Parts.")



The bulk measurement is the stochastic average of single cells

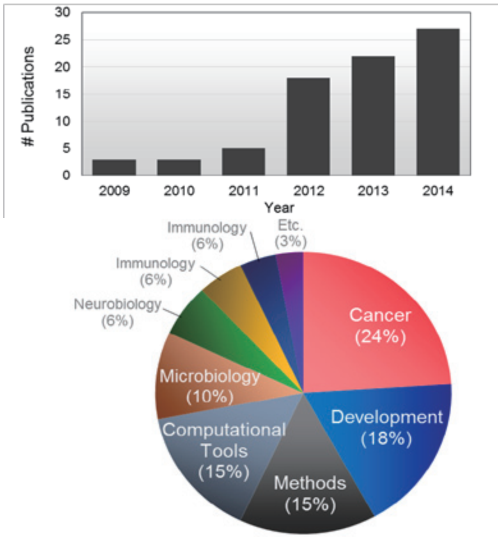


Kim KT, Lee HW, Lee HO et al., 2015 *Genome Biol.*

Single-cell analysis – a brief history

‘Single-cell sequencing’
Methods of the Year 2013

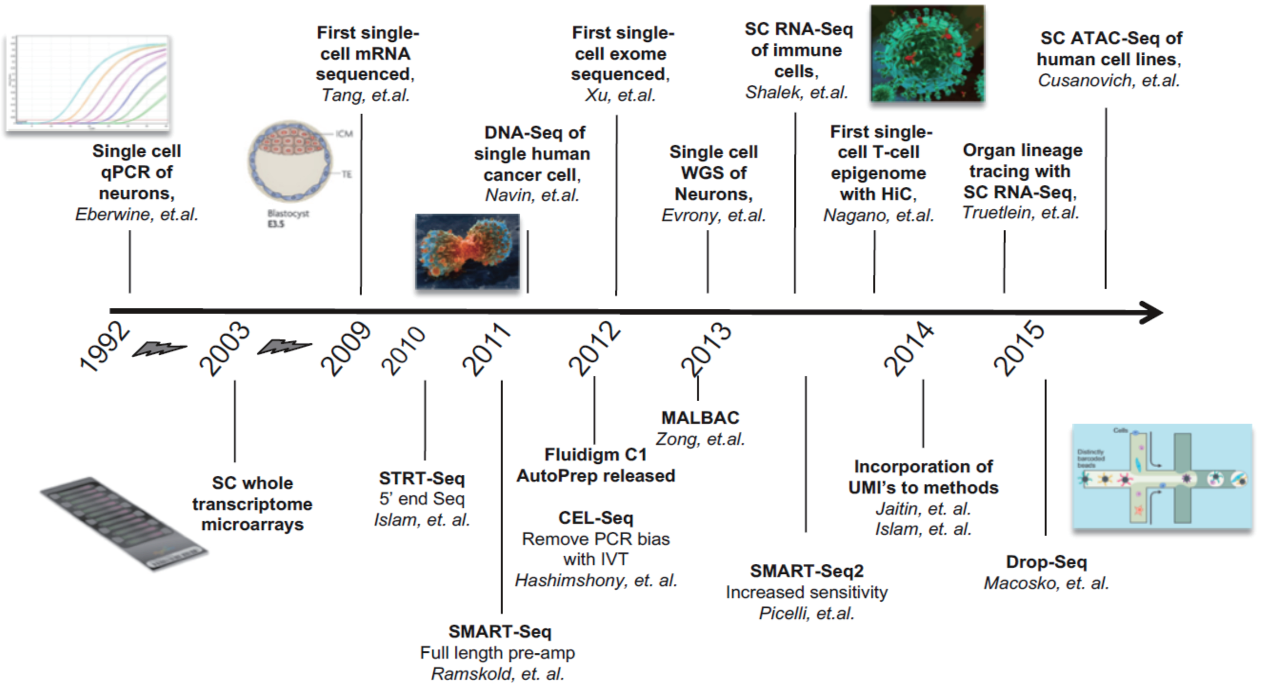
Rapid progress in
‘Single-cell sequencing’



adapted from Wang *et al. Mol Cell* 2015

[Tracking development cell by cell]
Breakthrough of the Year
2018 Science

Single-cell analysis – a brief history



1992 — **Single cell qPCR of neurons,** *Eberwine, et.al.*

2003 — **SC whole transcriptome microarrays**

2009 — **First single-cell mRNA sequenced,** *Tang, et.al.*

2010 — **STRT-Seq** 5' end Seq *Islam, et. al.*

2011 — **DNA-Seq of single human cancer cell,** *Navin, et.al.*

SMART-Seq Full length pre-amp *Ramskold, et. al.*

2012 — **First single-cell exome sequenced,** *Xu, et.al.*

Fluidigm C1 AutoPrep released

CEL-Seq Remove PCR bias with IVT *Hashimshony, et. al.*

2013 — **Single cell WGS of Neurons,** *Evrony, et.al.*

MALBAC *Zong, et.al.*

SC RNA-Seq of immune cells, *Shalek, et.al.*

SMART-Seq2 Increased sensitivity *Picelli, et.al.*

2014 — **First single-cell T-cell epigenome with HiC,** *Nagano, et.al.*

Incorporation of UMI's to methods *Jaitin, et. al.* *Islam, et. al.*

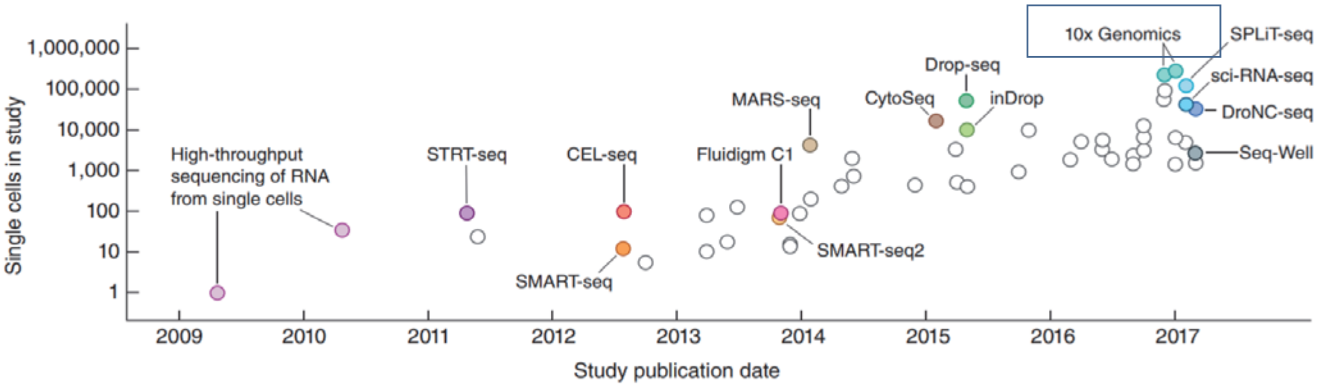
2015 — **Organ lineage tracing with SC RNA-Seq,** *Truetlein, et.al.*

Drop-Seq *Macosko, et. al.*

SC ATAC-Seq of human cell lines, *Cusanovich, et.al.*

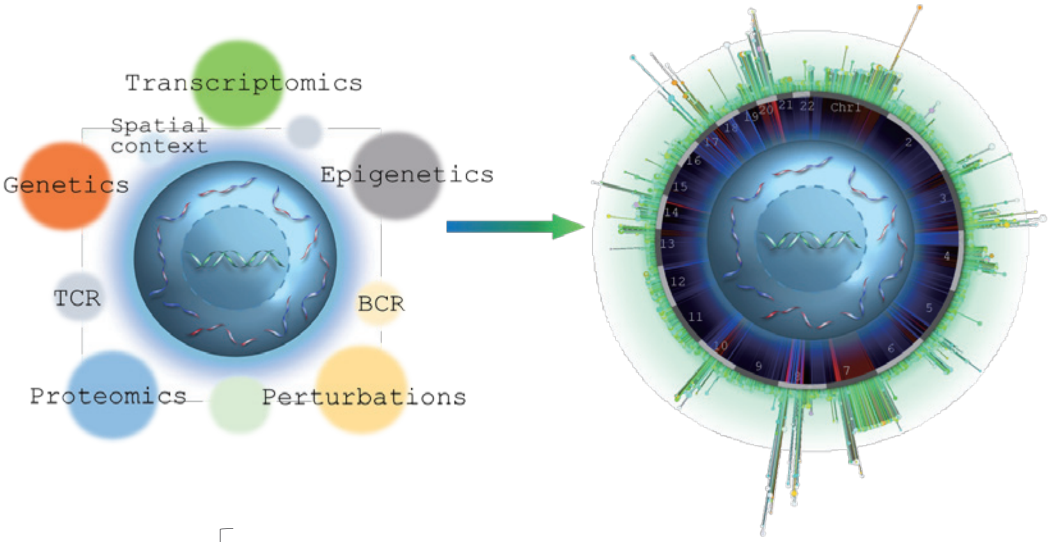
Kolodziejczyk, et. al. 2015; Navin, 2015

Trends: Increasing Dimensionality & More Cells



Sarah Teichmann group, 2018, *Nat Proc.*

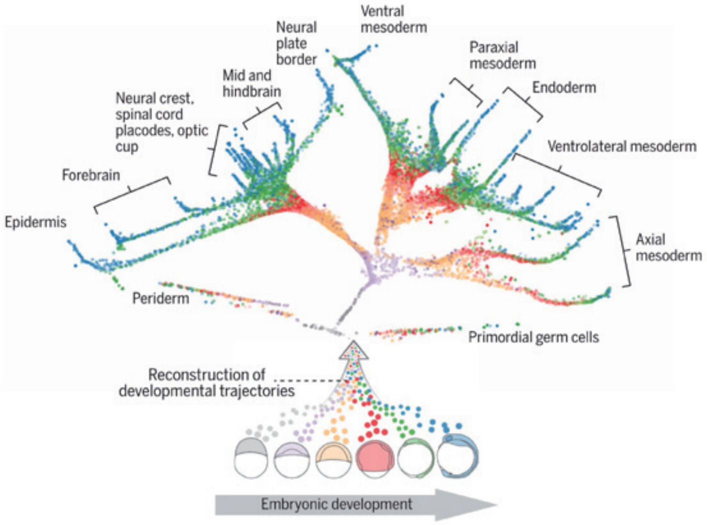
[Experimental Approaches]



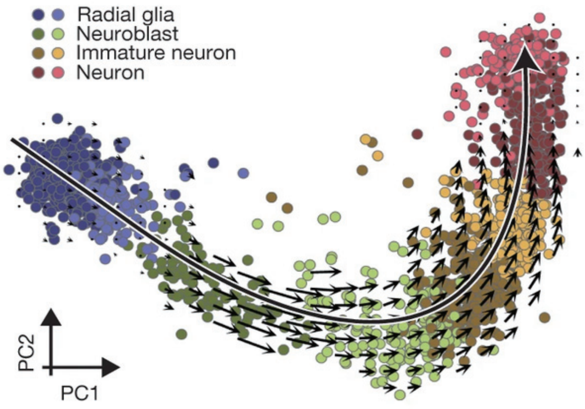
Multi-modal profiling methods at single-cell resolution

- DNA-seq + RNA-seq = **SIDR-seq**, **G&T-seq**, **DR-seq**
- DNA-seq + RNA-seq + Methyl-seq = **Trio-seq**
- RNA-seq + ATAC-seq = **sciCAR**
- RNA-seq + TCR/BCR = **(10X) 5'GEX with Immune Cell profiling**
- Epitope-profiling + RNA-seq + = **CITE-seq**
- Genotyping + RNA-seq = **GoT**
- Genetic screening with CRISPR + RNA-seq = **Perturb-seq**
- and......

[Computational Approaches]

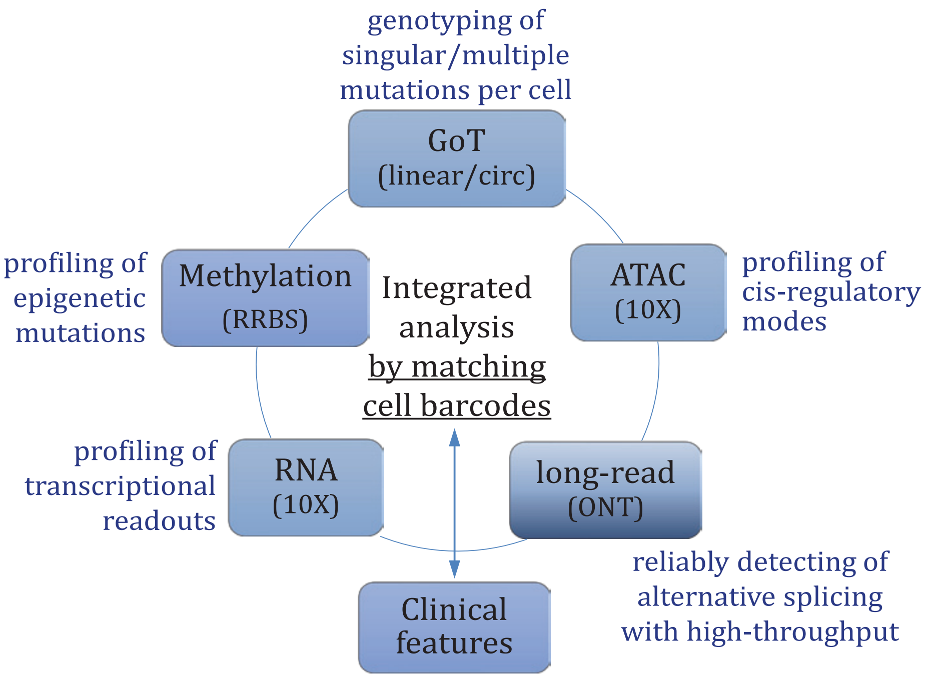


Farrell JA, Wang Y et al., 2018 *Science*



Manno GL et al., 2018 *Nature*

[Experimental & Computational Approaches]


genotyping of singular/multiple mutations per cell
GoT (linear/circ)
profiling of epigenetic mutations
Methylation (RRBS)
Integrated analysis by matching cell barcodes
ATAC (10X)
profiling of cis-regulatory modes
profiling of transcriptional readouts
RNA (10X)
long-read (ONT)
reliably detecting of alternative splicing with high-throughput
Clinical features

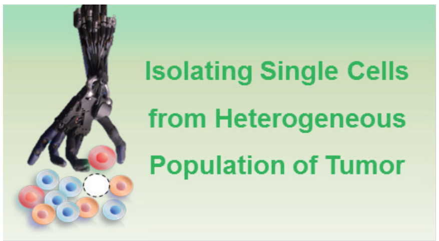
Highly Dimensional Single-cell Data Sets

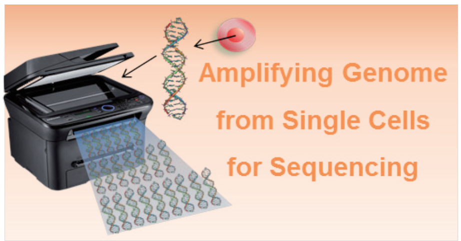
# Cells x **# Features** x **# Time Points** x **# Technologies**

dissected by

Sophisticated Analytical Design with Massive Computational Power

Basic single-cell analysis workflow





- Micropipetting
- Laser capture microdissection
- FACS
- Microfluidic circuits
- Droplet-based microfluidics

(DNA)

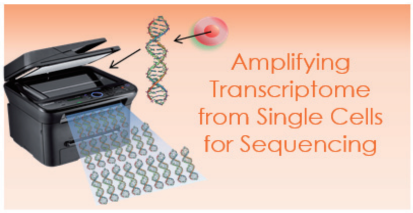
- MALBAC
- MDA
- LIANTI

(RNA)

- STRT-seq
- CEL-seq
- SMART/SMART2/SMART3-seq
- Droplet-based amplification (Drop-seq, inDrop, 10X)

(statistical/algorithmical mining)

At the initial stage of single-cell field



High-throughput sequencing
illumina
RNA sequencing

96 wells

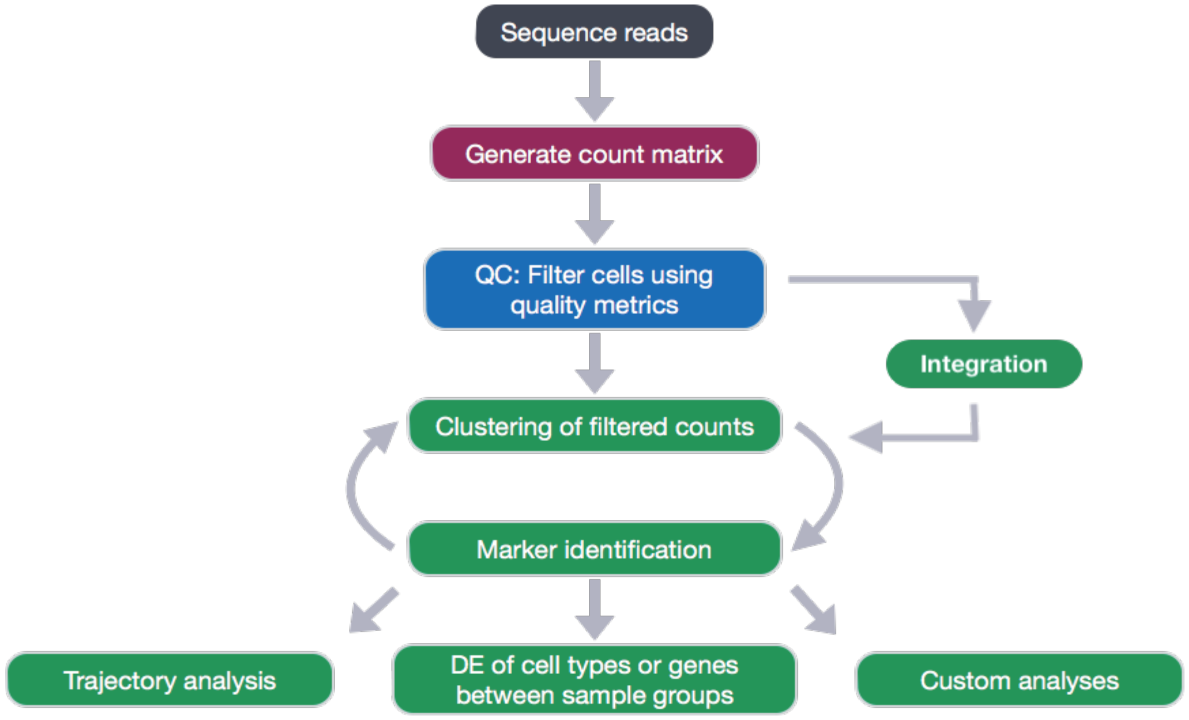
↓ Dead or live?
Singlet or doublet?

40-80 live cells captured

↓ How many genes detected?
How many reads aligned?
Mitochondria fraction?

20-60 cells of QC-passed

Basic data processing workflow



Sequence reads

Generate count matrix

QC: Filter cells using quality metrics

Integration

Clustering of filtered counts

Marker identification

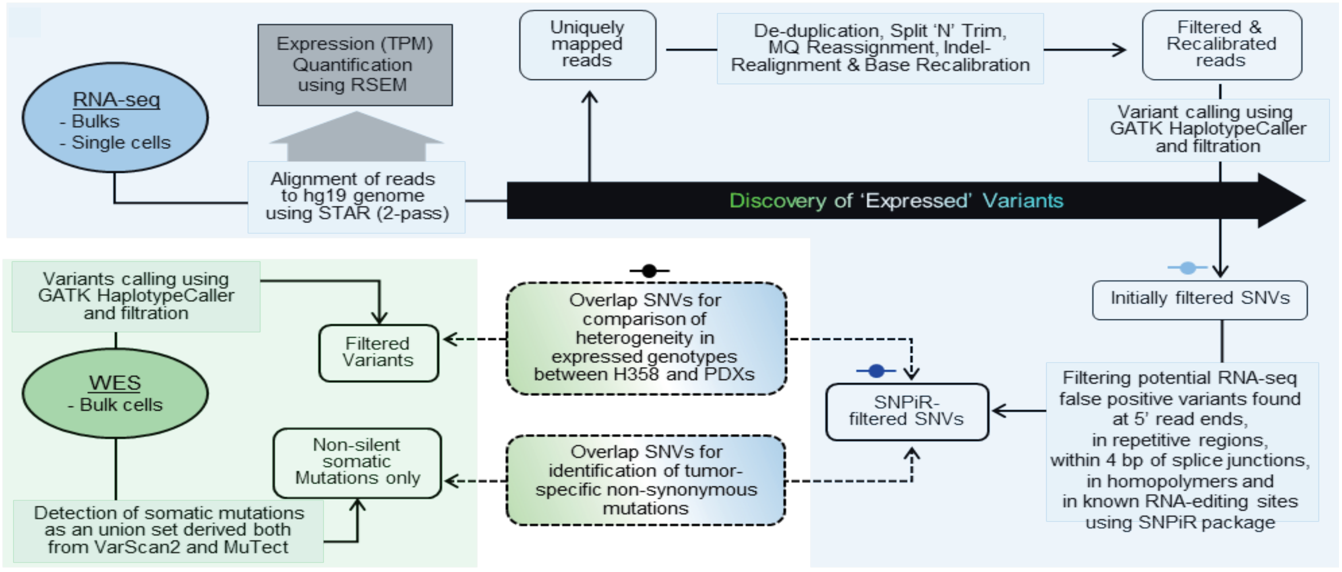
Trajectory analysis

DE of cell types or genes between sample groups

Custom analyses

Basic data processing workflow

for full-length/high-depth of several single-cells



RNA-seq
- Bulks
- Single cells

Expression (TPM) Quantification using RSEM

Alignment of reads to hg19 genome using STAR (2-pass)

Uniquely mapped reads

De-duplication, Split 'N' Trim, MQ Reassignment, Indel-Realignment & Base Recalibration

Filtered & Recalibrated reads

Variant calling using GATK HaplotypeCaller and filtration

Discovery of 'Expressed' Variants

Initially filtered SNVs

Filtering potential RNA-seq false positive variants found at 5' read ends, in repetitive regions, within 4 bp of splice junctions, in homopolymers and in known RNA-editing sites using SNPiR package

SNPiR-filtered SNVs

Overlap SNVs for comparison of heterogeneity in expressed genotypes between H358 and PDXs

Overlap SNVs for identification of tumor-specific non-synonymous mutations

Variants calling using GATK HaplotypeCaller and filtration

Filtered Variants

WES
- Bulk cells

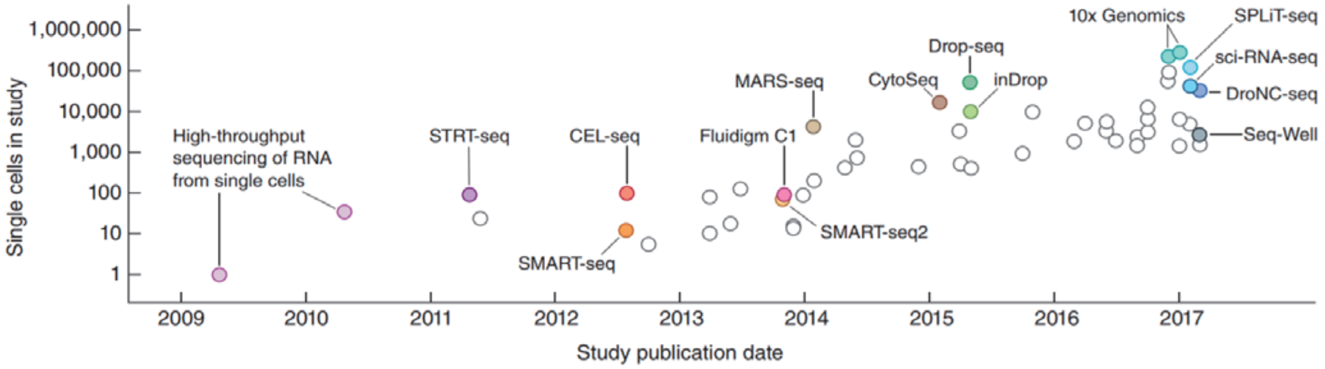
Non-silent somatic Mutations only

Detection of somatic mutations as an union set derived both from VarScan2 and MuTect

Kim KT, Lee HW, Lee HO et al., 2015 *Genome Biol.*

Trends: Increasing Dimensionality & More Cells

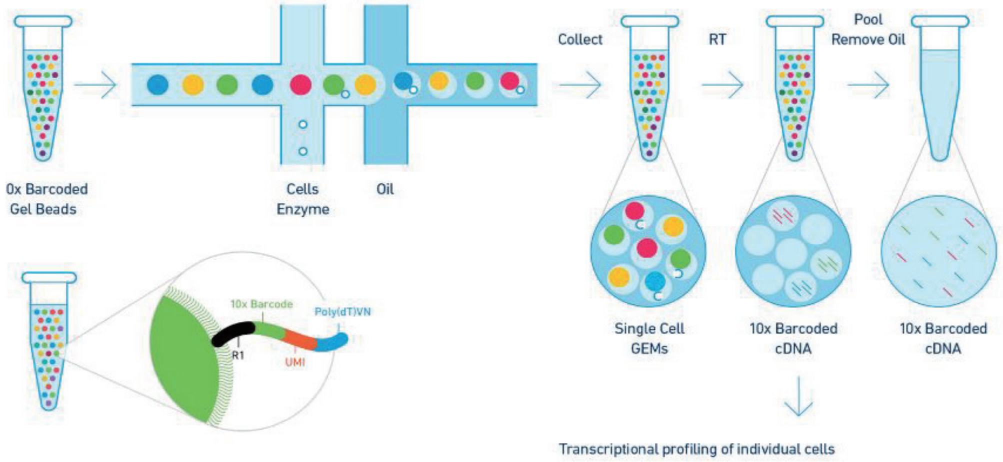
HUMAN CELL ATLAS



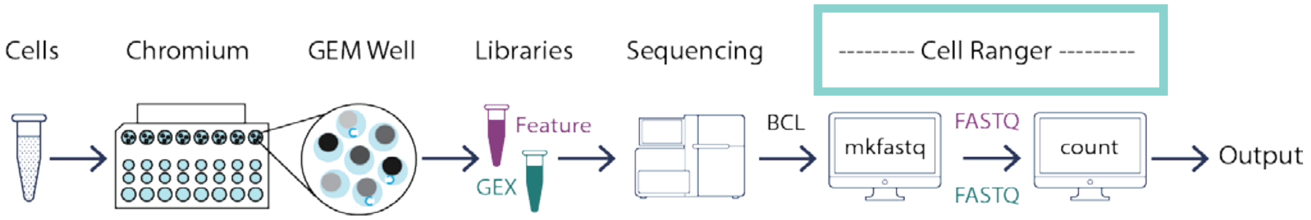
Sarah Teichmann group, 2018, *Nat Proc*.

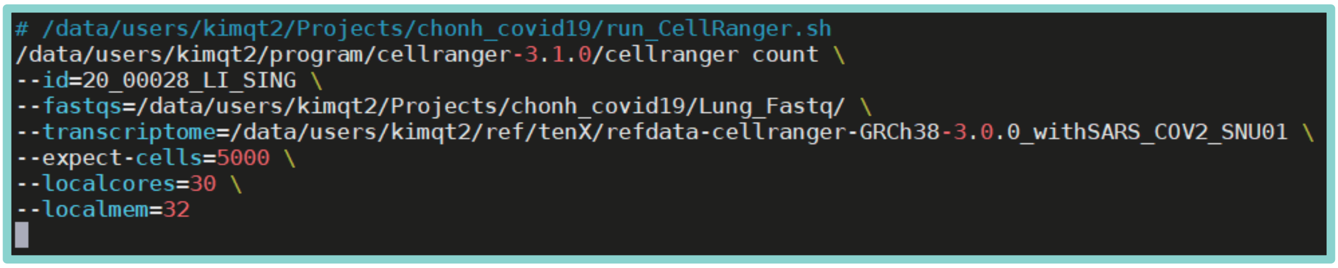
Basic single-cell analysis workflow

10X GENOMICS



Pre-processing pipeline: 10X CellRanger





```
# /data/users/kimqt2/Projects/chonh_covid19/run_CellRanger.sh
/data/users/kimqt2/program/cellranger-3.1.0/cellranger count \
--id=20_00028_LI_SING \
--fastqs=/data/users/kimqt2/Projects/chonh_covid19/Lung_Fastq/ \
--transcriptome=/data/users/kimqt2/ref/tenX/refdata-cellranger-GRCh38-3.0.0_withSARS_COV2_SNU01 \
--expect-cells=5000 \
--localcores=30 \
--localmem=32
```

--> Output: Gene-level expression matrix per cell

CellRanger (10X Genomics)

1. Read Trimming

> Detection/trimming of technically-induced sequence (TSO, template switch oligo)

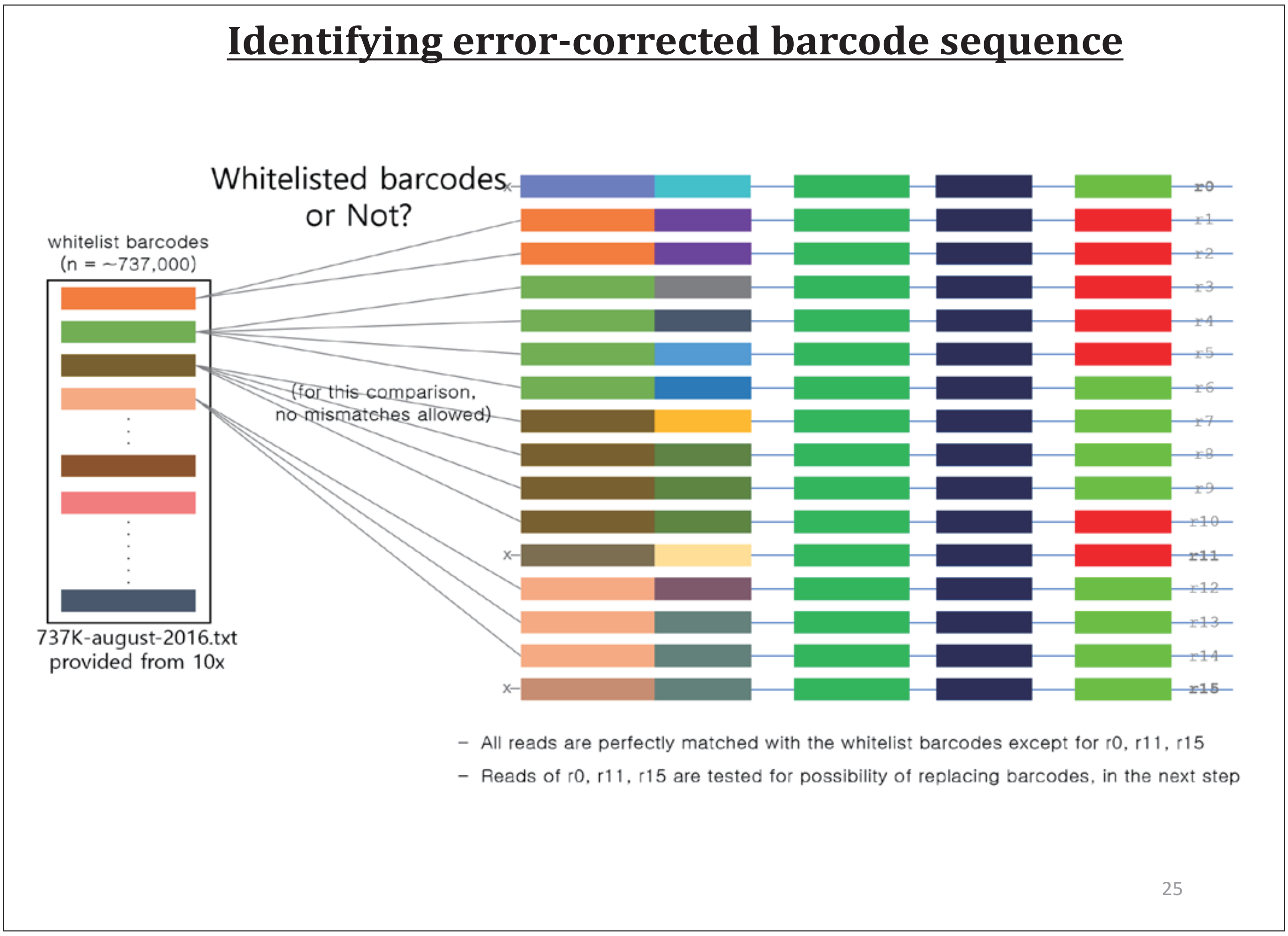
2. Read Alignment

> Splicing-aware alignment of cDNA sequences to the genome reference using STAR

3. Calling cell barcodes and UMI

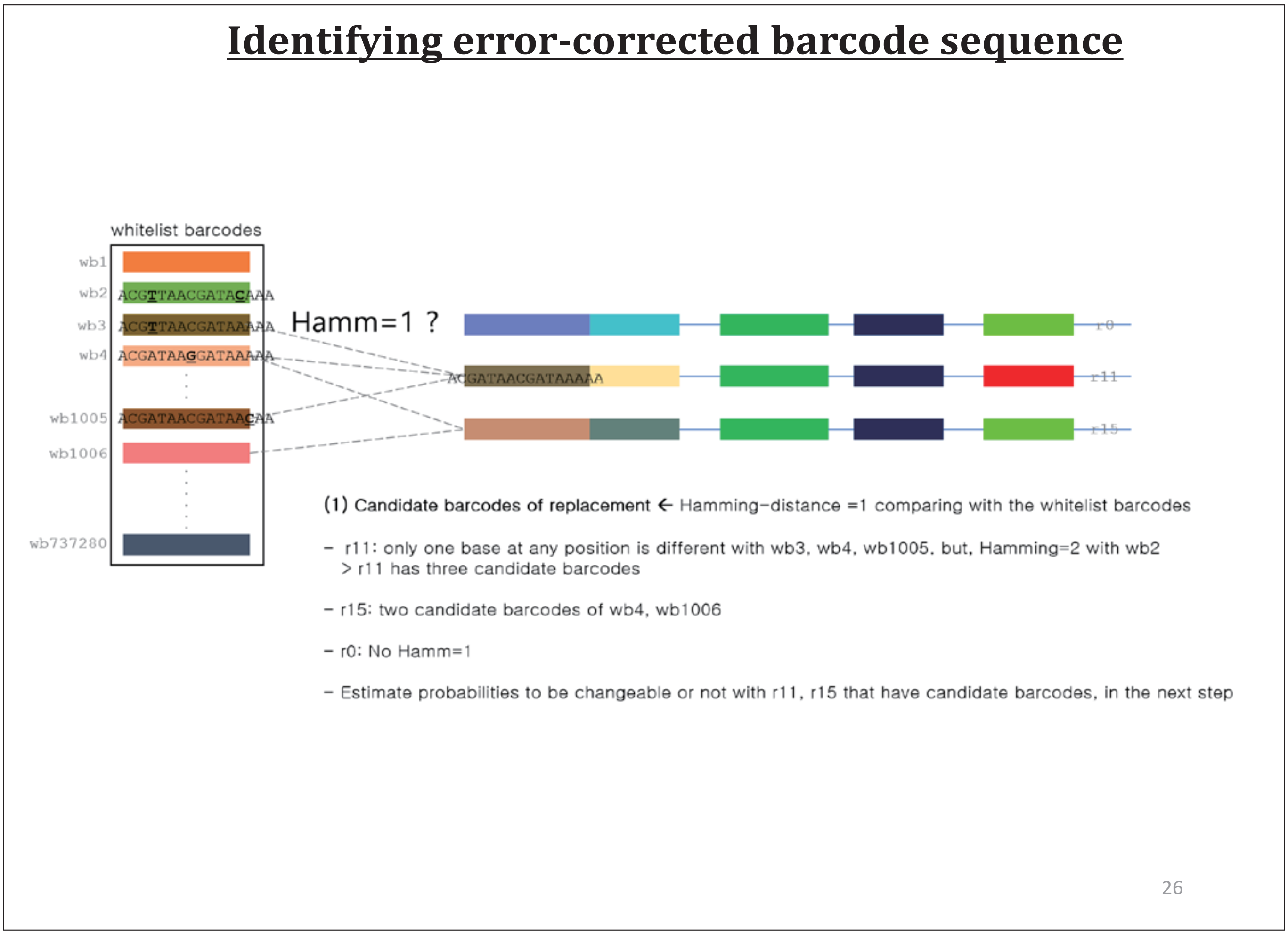
> Error-aware statistical correction of barcodes and UMI

4. Basic subclustering and dimensional reduction



Identifying error-corrected barcode sequence

- All reads are perfectly matched with the whitelist barcodes except for r0, r11, r15
- Reads of r0, r11, r15 are tested for possibility of replacing barcodes, in the next step



Identifying error-corrected barcode sequence

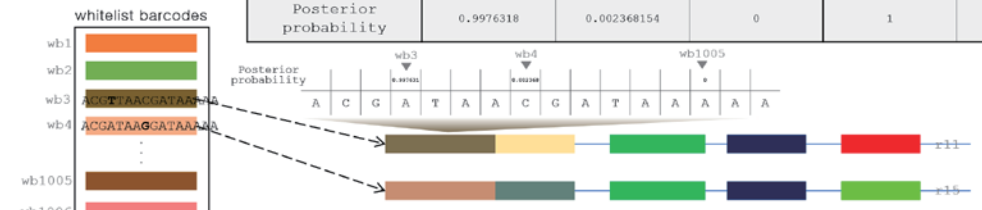
(1) Candidate barcodes of replacement ← Hamming-distance =1 comparing with the whitelist barcodes

- r11: only one base at any position is different with wb3, wb4, wb1005, but, Hamming=2 with wb2
  > r11 has three candidate barcodes
- r15: two candidate barcodes of wb4, wb1006
- r0: No Hamm=1
- Estimate probabilities to be changeable or not with r11, r15 that have candidate barcodes, in the next step

Identifying error-corrected barcode sequence

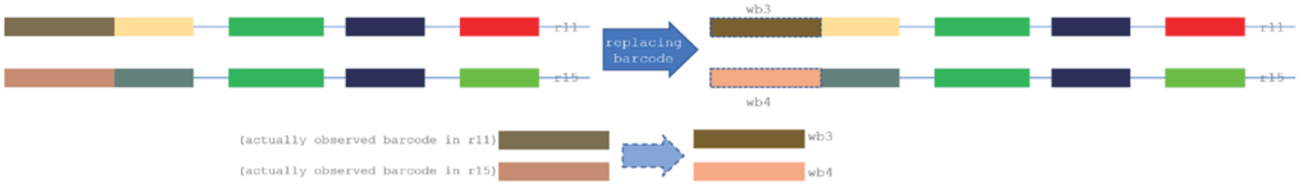
	r11			r15	
candidate barcodes	wb3	wb4	wb1005	wb4	wb1006
Posterior probability	0.9976318	0.002368154	0	1	0

Statistically Reliable to be replaced?

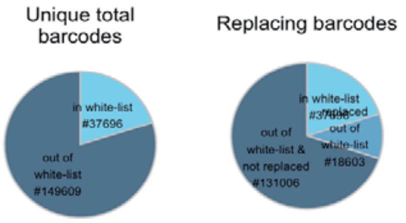


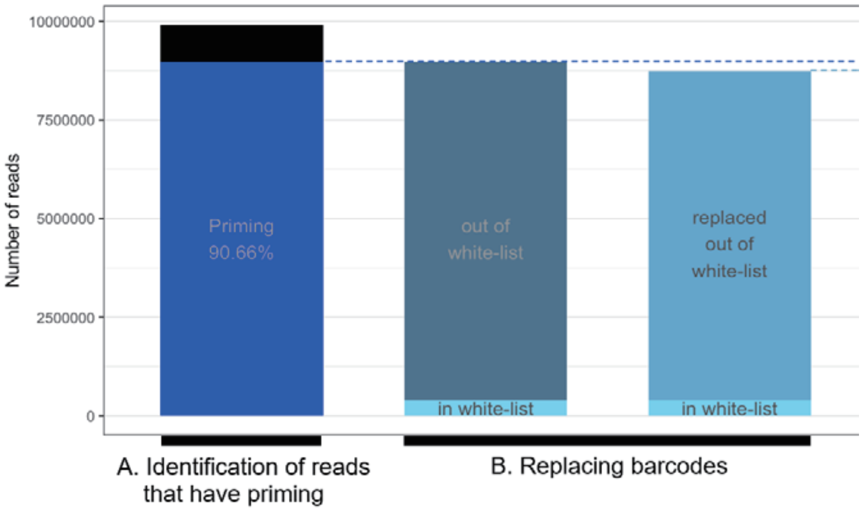
(3) supporting reads + base quality

> posterior probability >=0.99 --> Barcode replacement!
- r11: among candidate barcodes of wb3, wb4, wb1005, wb3 has the highest posterior probability (= 0.9976318)
  > if this maximum posterior probability >=0.99, then replace the observed barcode with this wb3
- r15: between candidate barcodes of wb4, wb1006, wb4 has the maximum posterior probability (= 1)
  > if this maximum posterior probability >=0.99, then replace the observed barcode with this wb4
- If the maximum posterior probability <0.99, regarded as not significant to be replaced of barcode and rescued of the read


r11
r15
replacing barcode
wb3
r11
r15
wb4
(actually observed barcode in r11)
(actually observed barcode in r15)
wb3
wb4

Identifying error-corrected barcode sequence





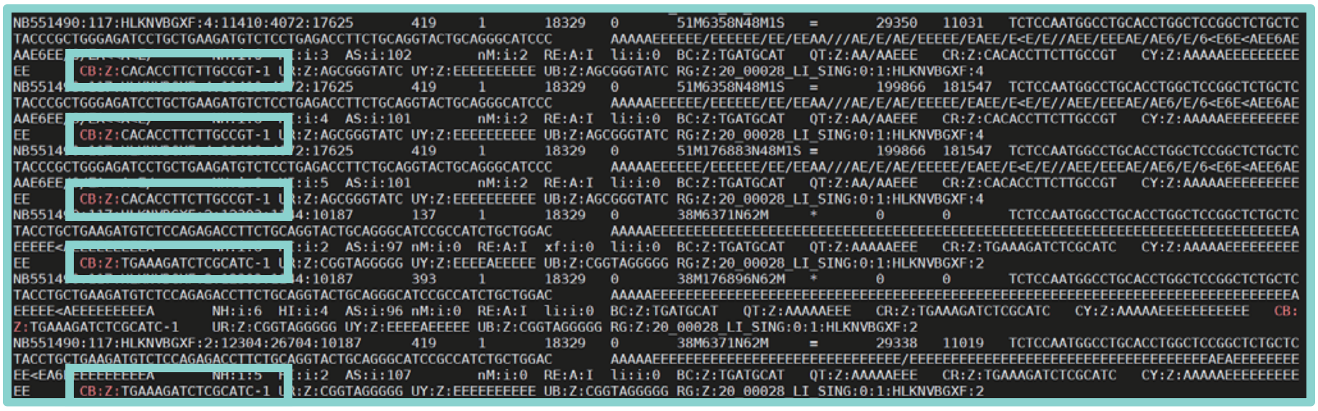
Output BAM

(White-listed barcodes)

- 10X v2.chemistry: `737K-august-2016.txt`
- 10X v3.chemistry: `3M-february-2018.txt`

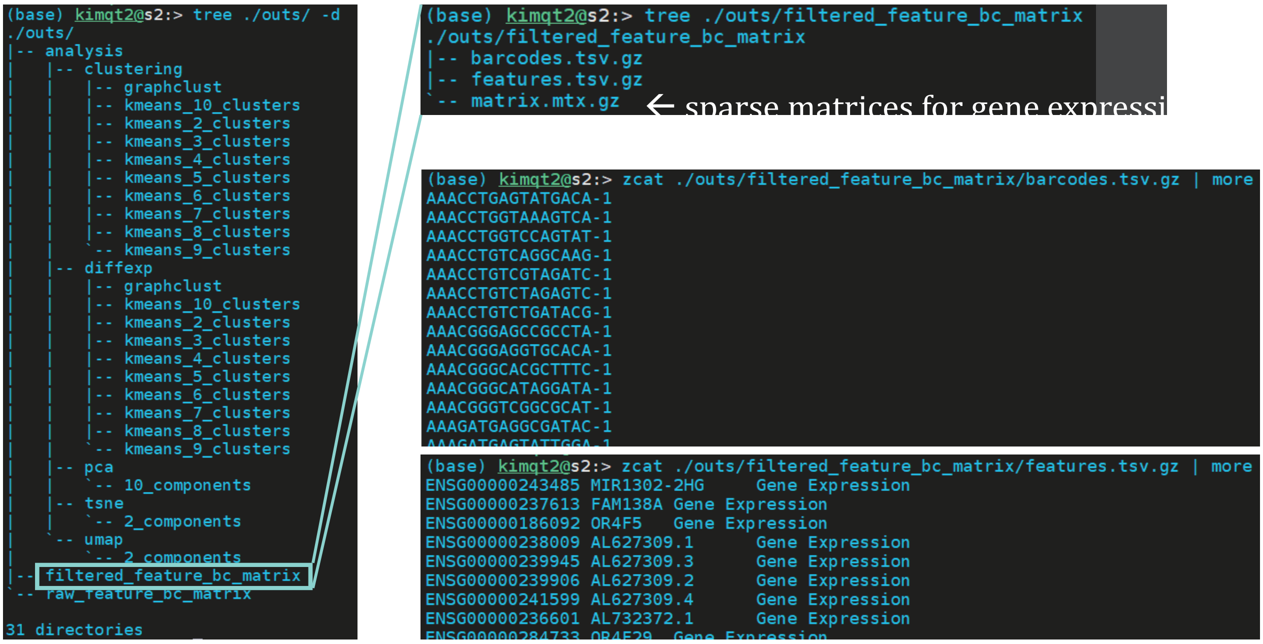
(Length of UMI)

- 10X v2.chemistry: `10`
- 10X v3.chemistry: `12`



```
NB551490:117:HLKNVBGXF:4:11410:4072:17625        419     1       18329   0       51M6358N48M1S   =       29350   11031   TCTCCAATGGCCTGCACCTGGCTCCGGCTCTGCTC
TACCCGCTGGGAGATCCTGCTGAAGATGTCTCCTGAGACCTTCTGCAGGTACTGCAGGGCATCCC       AAAAAEEEEEE/EEEEEE/EE/EEAA///AE/E/AE/EEEEE/EAEE/E<E/E//AEE/EEEAE/AE6/E/6<E6E<AEE6AE
AAE6EE,                       :i:3  AS:i:102         nM:i:2  RE:A:I  li:i:0  BC:Z:TGATGCAT   QT:Z:AA/AAEEE   CR:Z:CACACCTTCTTGCCGT   CY:Z:AAAAAEEEEEEEEEE
EE      CB:Z:CACACCTTCTTGCCGT-1 UR:Z:AGCGGGTATC UY:Z:EEEEEEEEEE UB:Z:AGCGGGTATC RG:Z:20_00028_LI_SING:0:1:HLKNVBGXF:4
NB5514                            2:17625        419     1       18329   0       51M6358N48M1S   =       199866  181547  TCTCCAATGGCCTGCACCTGGCTCCGGCTCTGCTC
TACCCGCTGGGAGATCCTGCTGAAGATGTCTCCTGAGACCTTCTGCAGGTACTGCAGGGCATCCC       AAAAAEEEEEE/EEEEEE/EE/EEAA///AE/E/AE/EEEEE/EAEE/E<E/E//AEE/EEEAE/AE6/E/6<E6E<AEE6AE
AAE6EE,                       :i:4  AS:i:101         nM:i:2  RE:A:I  li:i:0  BC:Z:TGATGCAT   QT:Z:AA/AAEEE   CR:Z:CACACCTTCTTGCCGT   CY:Z:AAAAAEEEEEEEEEE
EE      CB:Z:CACACCTTCTTGCCGT-1 UR:Z:AGCGGGTATC UY:Z:EEEEEEEEEE UB:Z:AGCGGGTATC RG:Z:20_00028_LI_SING:0:1:HLKNVBGXF:4
NB5514                            2:17625        419     1       18329   0       51M176883N48M1S =       199866  181547  TCTCCAATGGCCTGCACCTGGCTCCGGCTCTGCTC
TACCCGCTGGGAGATCCTGCTGAAGATGTCTCCTGAGACCTTCTGCAGGTACTGCAGGGCATCCC       AAAAAEEEEEE/EEEEEE/EE/EEAA///AE/E/AE/EEEEE/EAEE/E<E/E//AEE/EEEAE/AE6/E/6<E6E<AEE6AE
AAE6EE,                       :i:5  AS:i:101         nM:i:2  RE:A:I  li:i:0  BC:Z:TGATGCAT   QT:Z:AA/AAEEE   CR:Z:CACACCTTCTTGCCGT   CY:Z:AAAAAEEEEEEEEEE
EE      CB:Z:CACACCTTCTTGCCGT-1 UR:Z:AGCGGGTATC UY:Z:EEEEEEEEEE UB:Z:AGCGGGTATC RG:Z:20_00028_LI_SING:0:1:HLKNVBGXF:4
NB5514                            4:10187        137     1       18329   0       38M6371N62M     *       0       0       TCTCCAATGGCCTGCACCTGGCTCCGGCTCTGCTC
TACCTGCTGAAGATGTCTCCAGAGACCTTCTGCAGGTACTGCAGGGCATCCGCCATCTGCTGGAC       AAAAAEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEA
EEEEE<                        :i:2  AS:i:97 nM:i:0  RE:A:I  xf:i:0  li:i:0  BC:Z:TGATGCAT   QT:Z:AAAAAEEE   CR:Z:TGAAAGATCTCGCATC   CY:Z:AAAAAEEEEEEEEEE
EE      CB:Z:TGAAAGATCTCGCATC-1 UR:Z:CGGTAGGGGG UY:Z:EEEEAEEEEE UB:Z:CGGTAGGGGG RG:Z:20_00028_LI_SING:0:1:HLKNVBGXF:2
NB5514                            4:10187        393     1       18329   0       38M176896N62M   *       0       0       TCTCCAATGGCCTGCACCTGGCTCCGGCTCTGCTC
TACCTGCTGAAGATGTCTCCAGAGACCTTCTGCAGGTACTGCAGGGCATCCGCCATCTGCTGGAC       AAAAAEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEA
EEEEE<AEEEEEEEEEA       NH:i:6  HI:i:4  AS:i:96 nM:i:0  RE:A:I  li:i:0  BC:Z:TGATGCAT   QT:Z:AAAAAEEE   CR:Z:TGAAAGATCTCGCATC   CY:Z:AAAAAEEEEEEEEEE   CB:
Z:TGAAAGATCTCGCATC-1    UR:Z:CGGTAGGGGG UY:Z:EEEEAEEEEE UB:Z:CGGTAGGGGG RG:Z:20_00028_LI_SING:0:1:HLKNVBGXF:2
NB551490:117:HLKNVBGXF:2:12304:26704:10187       419     1       18329   0       38M6371N62M     =       29338   11019   TCTCCAATGGCCTGCACCTGGCTCCGGCTCTGCTC
TACCTGCTGAAGATGTCTCCAGAGACCTTCTGCAGGTACTGCAGGGCATCCGCCATCTGCTGGAC       AAAAAEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEE/EEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEAEAEEEEEEEE
EE<EA6                  NH:i:5    :i:2  AS:i:107         nM:i:0  RE:A:I  li:i:0  BC:Z:TGATGCAT   QT:Z:AAAAAEEE   CR:Z:TGAAAGATCTCGCATC   CY:Z:AAAAAEEEEEEEEEE
EE      CB:Z:TGAAAGATCTCGCATC-1 UR:Z:CGGTAGGGGG UY:Z:EEEEEEEEEE UB:Z:CGGTAGGGGG RG:Z:20_00028_LI_SING:0:1:HLKNVBGXF:2
```

Output matrices



```
(base) kimqt2@s2:> tree ./outs/ -d
./outs/
|-- analysis
|   |-- clustering
|   |   |-- graphclust
|   |   |-- kmeans_10_clusters
|   |   |-- kmeans_2_clusters
|   |   |-- kmeans_3_clusters
|   |   |-- kmeans_4_clusters
|   |   |-- kmeans_5_clusters
|   |   |-- kmeans_6_clusters
|   |   |-- kmeans_7_clusters
|   |   |-- kmeans_8_clusters
|   |   `-- kmeans_9_clusters
|   |-- diffexp
|   |   |-- graphclust
|   |   |-- kmeans_10_clusters
|   |   |-- kmeans_2_clusters
|   |   |-- kmeans_3_clusters
|   |   |-- kmeans_4_clusters
|   |   |-- kmeans_5_clusters
|   |   |-- kmeans_6_clusters
|   |   |-- kmeans_7_clusters
|   |   |-- kmeans_8_clusters
|   |   `-- kmeans_9_clusters
|   |-- pca
|   |   `-- 10_components
|   |-- tsne
|   |   `-- 2_components
|   `-- umap
|       `-- 2_components
|-- filtered_feature_bc_matrix
`-- raw_feature_bc_matrix

31 directories
```

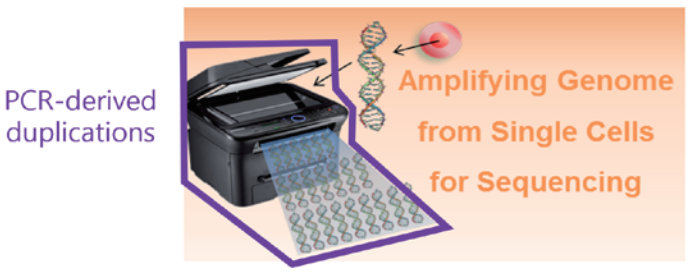
```
(base) kimqt2@s2:> tree ./outs/filtered_feature_bc_matrix
./outs/filtered_feature_bc_matrix
|-- barcodes.tsv.gz
|-- features.tsv.gz
`-- matrix.mtx.gz
```

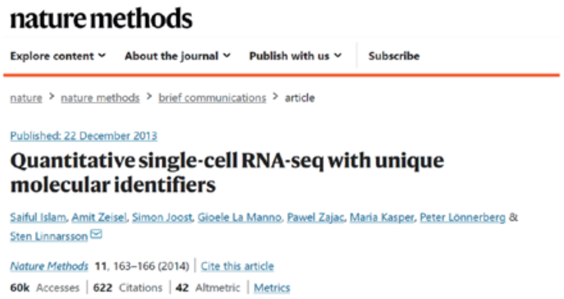
← sparse matrices for gene expressi

```
(base) kimqt2@s2:> zcat ./outs/filtered_feature_bc_matrix/barcodes.tsv.gz | more
AAACCTGAGTATGACA-1
AAACCTGGTAAAGTCA-1
AAACCTGGTCCAGTAT-1
AAACCTGTCAGGCAAG-1
AAACCTGTCGTAGATC-1
AAACCTGTCTAGAGTC-1
AAACCTGTCTGATACG-1
AAACGGGAGCCGCCTA-1
AAACGGGAGGTGCACA-1
AAACGGGCACGCTTTC-1
AAACGGGCATAGGATA-1
AAACGGGTCGGCGCAT-1
AAAGATGAGGCGATAC-1
AAAGATGAGTATTGGA-1
```

```
(base) kimqt2@s2:> zcat ./outs/filtered_feature_bc_matrix/features.tsv.gz | more
ENSG00000243485 MIR1302-2HG     Gene Expression
ENSG00000237613 FAM138A Gene Expression
ENSG00000186092 OR4F5   Gene Expression
ENSG00000238009 AL627309.1      Gene Expression
ENSG00000239945 AL627309.3      Gene Expression
ENSG00000239906 AL627309.2      Gene Expression
ENSG00000241599 AL627309.4      Gene Expression
ENSG00000236601 AL732372.1      Gene Expression
ENSG00000284733 OR4F29  Gene Expression
```

Estimation of relative level of gene expression & Normalization of their abundances





nature methods

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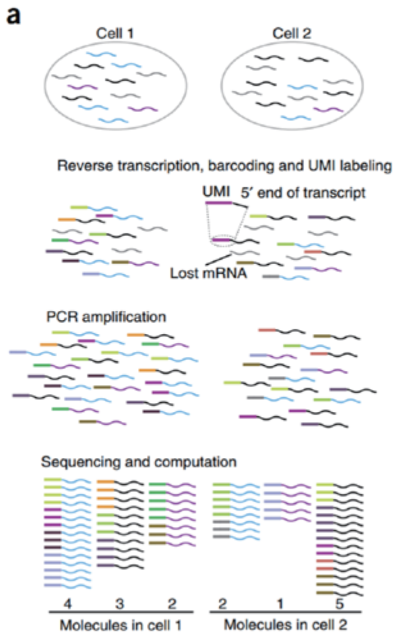
Published: 22 December 2013

Quantitative single-cell RNA-seq with unique molecular identifiers

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

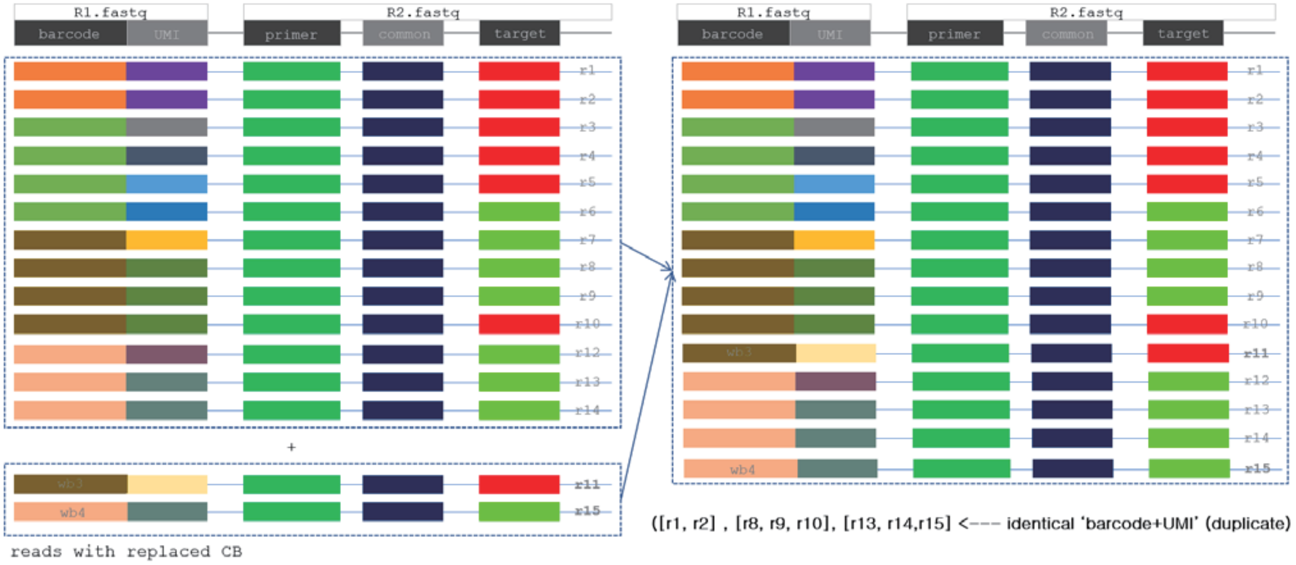
Nature Methods 11, 163–166 (2014) | Cite this article

60k Accesses | 622 Citations | 42 Altmetric | Metrics



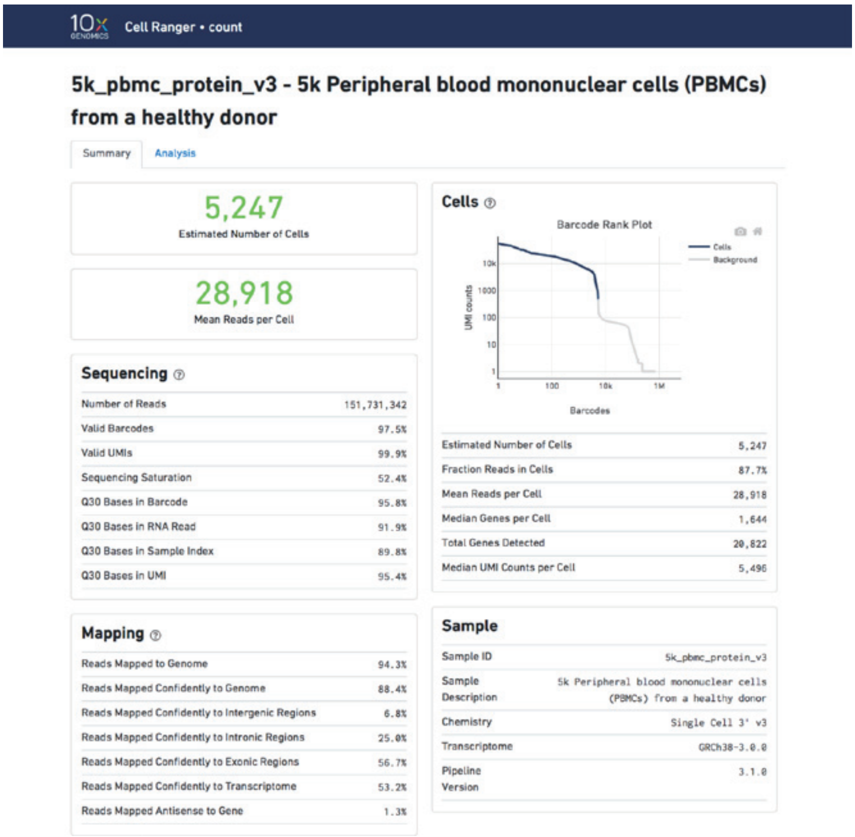
duplication을 구별하기 위해서 Unique sequence tagging: UMI (Unique Molecular Identifier)

Estimation of relative level of gene expression & Normalization of their abundances



([r1, r2] , [r8, r9, r10], [r13, r14,r15] <--- identical 'barcode+UMI' (duplicate)

Output matrices



5k_pbmc_protein_v3 - 5k Peripheral blood mononuclear cells (PBMCs) from a healthy donor

5,247
Estimated Number of Cells

28,918
Mean Reads per Cell

Sequencing

Number of Reads	151,731,342
Valid Barcodes	97.5%
Valid UMIs	99.9%
Sequencing Saturation	52.4%
Q30 Bases in Barcode	95.8%
Q30 Bases in RNA Read	91.9%
Q30 Bases in Sample Index	89.8%
Q30 Bases in UMI	95.4%

Mapping

Reads Mapped to Genome	94.3%
Reads Mapped Confidently to Genome	88.4%
Reads Mapped Confidently to Intergenic Regions	6.8%
Reads Mapped Confidently to Intronic Regions	25.0%
Reads Mapped Confidently to Exonic Regions	56.7%
Reads Mapped Confidently to Transcriptome	53.2%
Reads Mapped Antisense to Gene	1.3%

Cells

Estimated Number of Cells	5,247
Fraction Reads in Cells	87.7%
Mean Reads per Cell	28,918
Median Genes per Cell	1,644
Total Genes Detected	20,822
Median UMI Counts per Cell	5,496

Sample

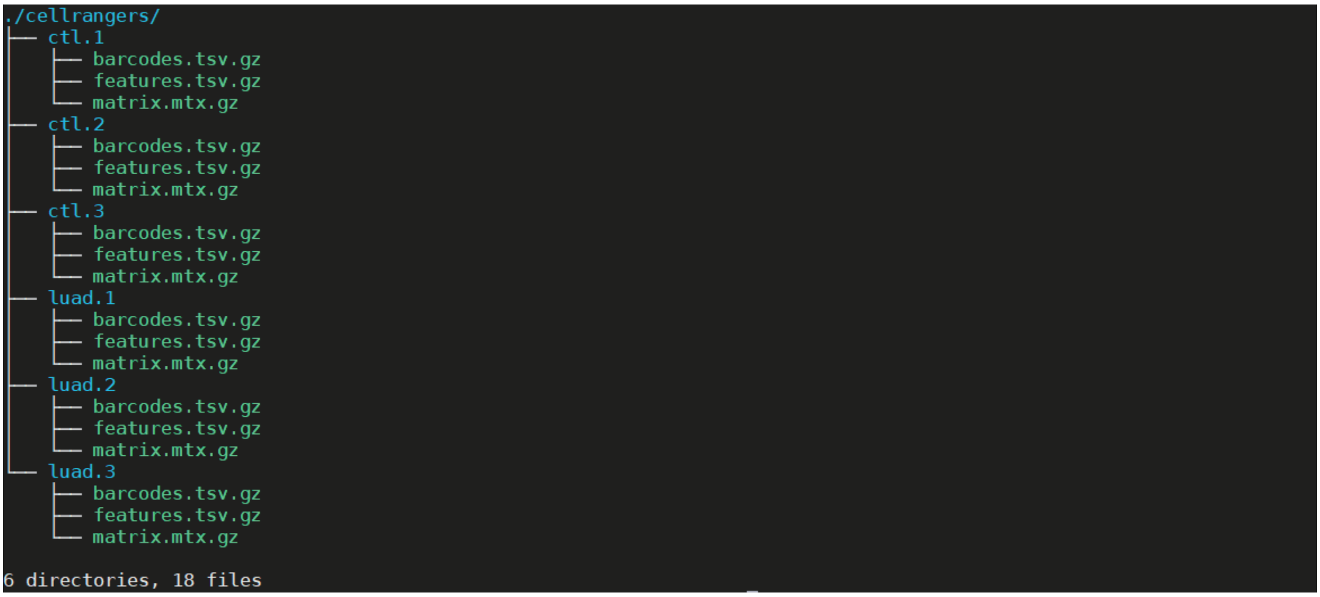
Sample ID	5k_pbmc_protein_v3
Sample Description	5k Peripheral blood mononuclear cells (PBMCs) from a healthy donor
Chemistry	Single Cell 3' v3
Transcriptome	GRCh38-3.0.0
Pipeline Version	3.1.0

Seurat R package:
the most popular tool package processing 10X output data

https://satijalab.org/seurat

1) Read the 10X output data
2) QC and select cells for further analysis
3) Normalize the data
4) Detection of variable genes across the single cells
5) Scale the data and remove unwanted sources of variation
6) Perform linear dimensional reduction
7) Determine statistically significant principal components
8) Cluster the cells
9) Run non-linear dimensional reduction
10) Find differentially expressed genes (cluster biomarkers)
11) Assign cell type identity to clusters
12) Further sub-dissect within cell types

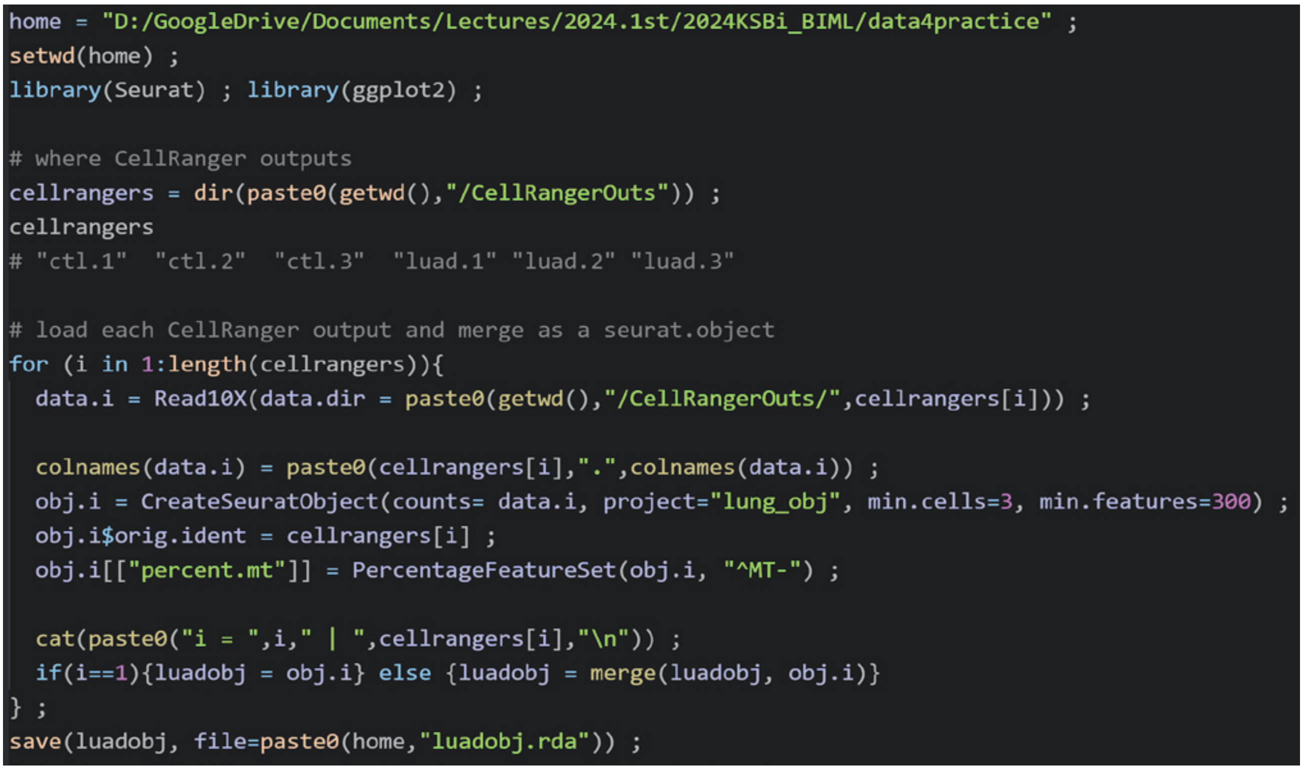
Data loading (practice)



```
./cellrangers/
├── ctl.1
│   ├── barcodes.tsv.gz
│   ├── features.tsv.gz
│   └── matrix.mtx.gz
├── ctl.2
│   ├── barcodes.tsv.gz
│   ├── features.tsv.gz
│   └── matrix.mtx.gz
├── ctl.3
│   ├── barcodes.tsv.gz
│   ├── features.tsv.gz
│   └── matrix.mtx.gz
├── luad.1
│   ├── barcodes.tsv.gz
│   ├── features.tsv.gz
│   └── matrix.mtx.gz
├── luad.2
│   ├── barcodes.tsv.gz
│   ├── features.tsv.gz
│   └── matrix.mtx.gz
└── luad.3
    ├── barcodes.tsv.gz
    ├── features.tsv.gz
    └── matrix.mtx.gz

6 directories, 18 files
```

Data loading



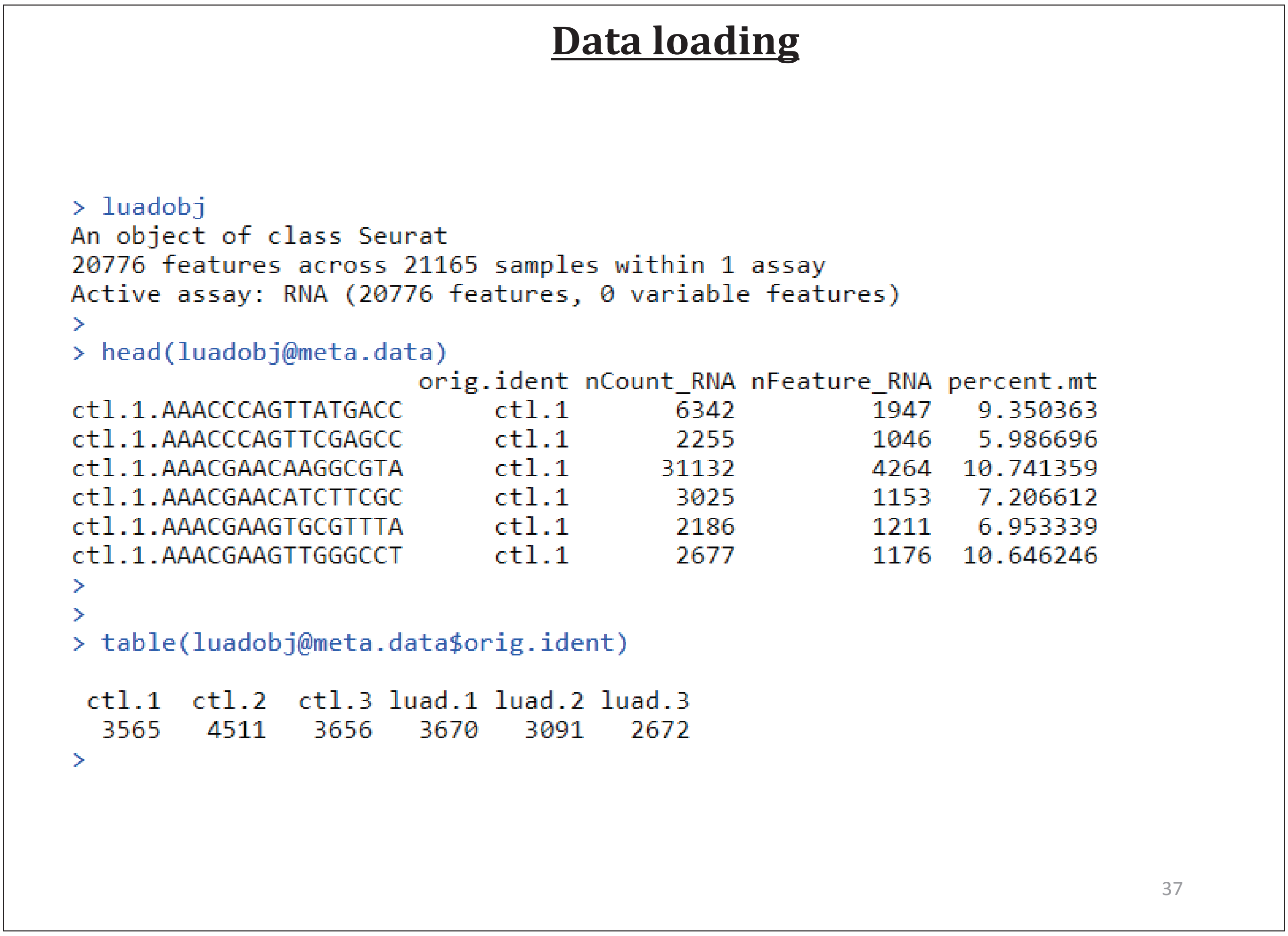
```
home = "D:/GoogleDrive/Documents/Lectures/2024.1st/2024KSBi_BIML/data4practice" ;
setwd(home) ;
library(Seurat) ; library(ggplot2) ;

# where CellRanger outputs
cellrangers = dir(paste0(getwd(),"/CellRangerOuts")) ;
cellrangers
# "ctl.1"  "ctl.2"  "ctl.3"  "luad.1" "luad.2" "luad.3"

# load each CellRanger output and merge as a seurat.object
for (i in 1:length(cellrangers)){
  data.i = Read10X(data.dir = paste0(getwd(),"/CellRangerOuts/",cellrangers[i])) ;

  colnames(data.i) = paste0(cellrangers[i],".",colnames(data.i)) ;
  obj.i = CreateSeuratObject(counts= data.i, project="lung_obj", min.cells=3, min.features=300) ;
  obj.i$orig.ident = cellrangers[i] ;
  obj.i[["percent.mt"]] = PercentageFeatureSet(obj.i, "^MT-") ;

  cat(paste0("i = ",i," | ",cellrangers[i],"\n")) ;
  if(i==1){luadobj = obj.i} else {luadobj = merge(luadobj, obj.i)}
} ;
save(luadobj, file=paste0(home,"luadobj.rda")) ;
```

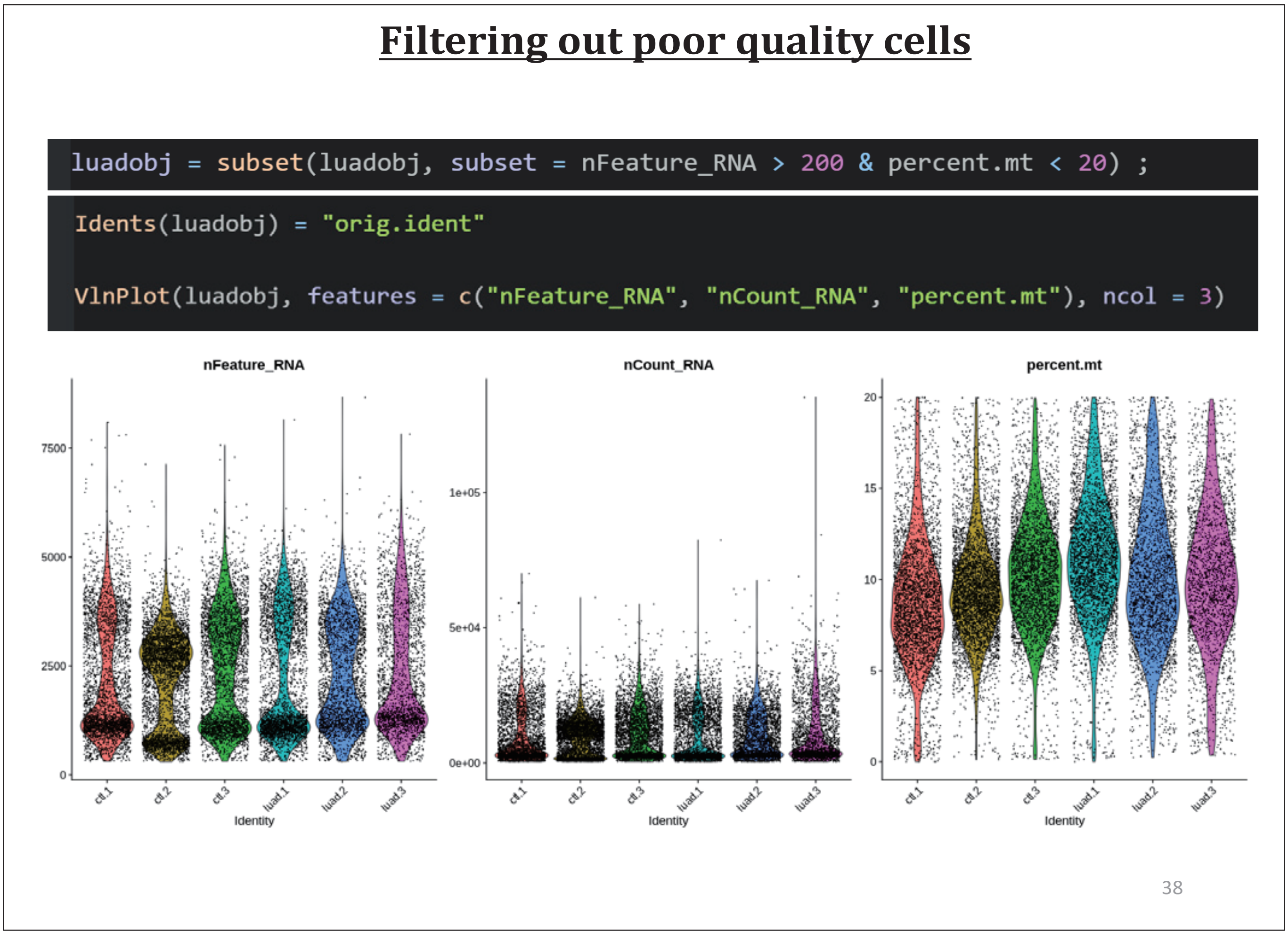


Data loading

```
> luadobj
An object of class Seurat 
20776 features across 21165 samples within 1 assay 
Active assay: RNA (20776 features, 0 variable features)
> 
> head(luadobj@meta.data)
                       orig.ident nCount_RNA nFeature_RNA percent.mt
ctl.1.AAACCCAGTTATGACC      ctl.1       6342         1947   9.350363
ctl.1.AAACCCAGTTCGAGCC      ctl.1       2255         1046   5.986696
ctl.1.AAACGAACAAGGCGTA      ctl.1      31132         4264  10.741359
ctl.1.AAACGAACATCTTCGC      ctl.1       3025         1153   7.206612
ctl.1.AAACGAAGTGCGTTTA      ctl.1       2186         1211   6.953339
ctl.1.AAACGAAGTTGGGCCT      ctl.1       2677         1176  10.646246
> 
> 
> table(luadobj@meta.data$orig.ident)

 ctl.1  ctl.2  ctl.3 luad.1 luad.2 luad.3 
  3565   4511   3656   3670   3091   2672 
> 
```

37



Filtering out poor quality cells

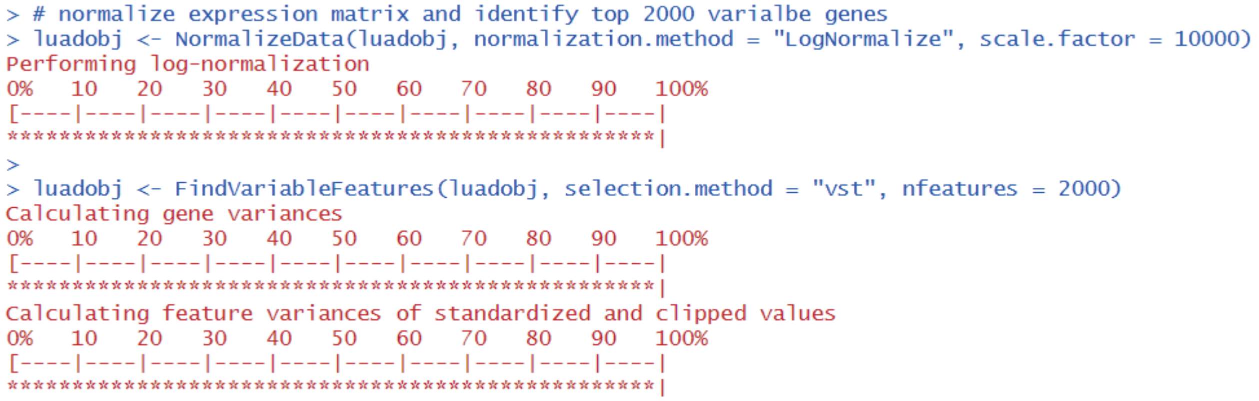
```
luadobj = subset(luadobj, subset = nFeature_RNA > 200 & percent.mt < 20) ;
```

```
Idents(luadobj) = "orig.ident"

VlnPlot(luadobj, features = c("nFeature_RNA", "nCount_RNA", "percent.mt"), ncol = 3)
```

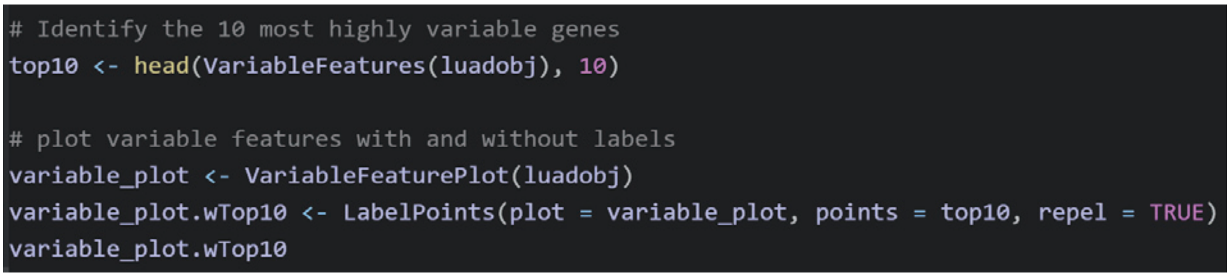
38

Normalize expression matrix and identify top variable genes



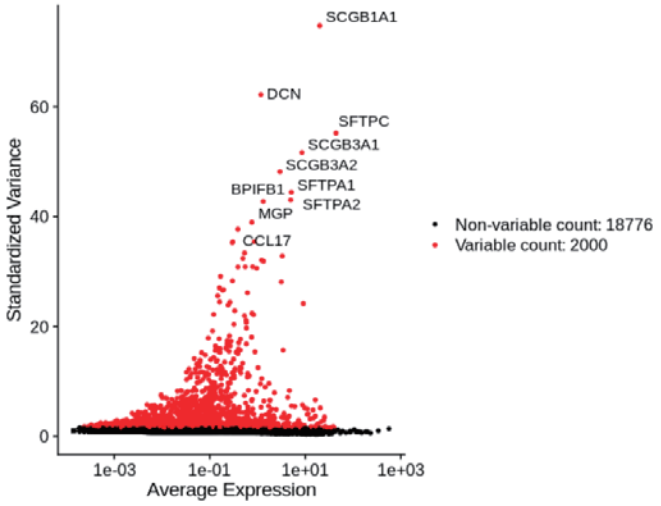
```
> # normalize expression matrix and identify top 2000 varialbe genes
> luadobj <- NormalizeData(luadobj, normalization.method = "LogNormalize", scale.factor = 10000)
Performing log-normalization
0%   10   20   30   40   50   60   70   80   90   100%
[----|----|----|----|----|----|----|----|----|----|
**************************************************|
>
> luadobj <- FindVariableFeatures(luadobj, selection.method = "vst", nfeatures = 2000)
Calculating gene variances
0%   10   20   30   40   50   60   70   80   90   100%
[----|----|----|----|----|----|----|----|----|----|
**************************************************|
Calculating feature variances of standardized and clipped values
0%   10   20   30   40   50   60   70   80   90   100%
[----|----|----|----|----|----|----|----|----|----|
**************************************************|
```

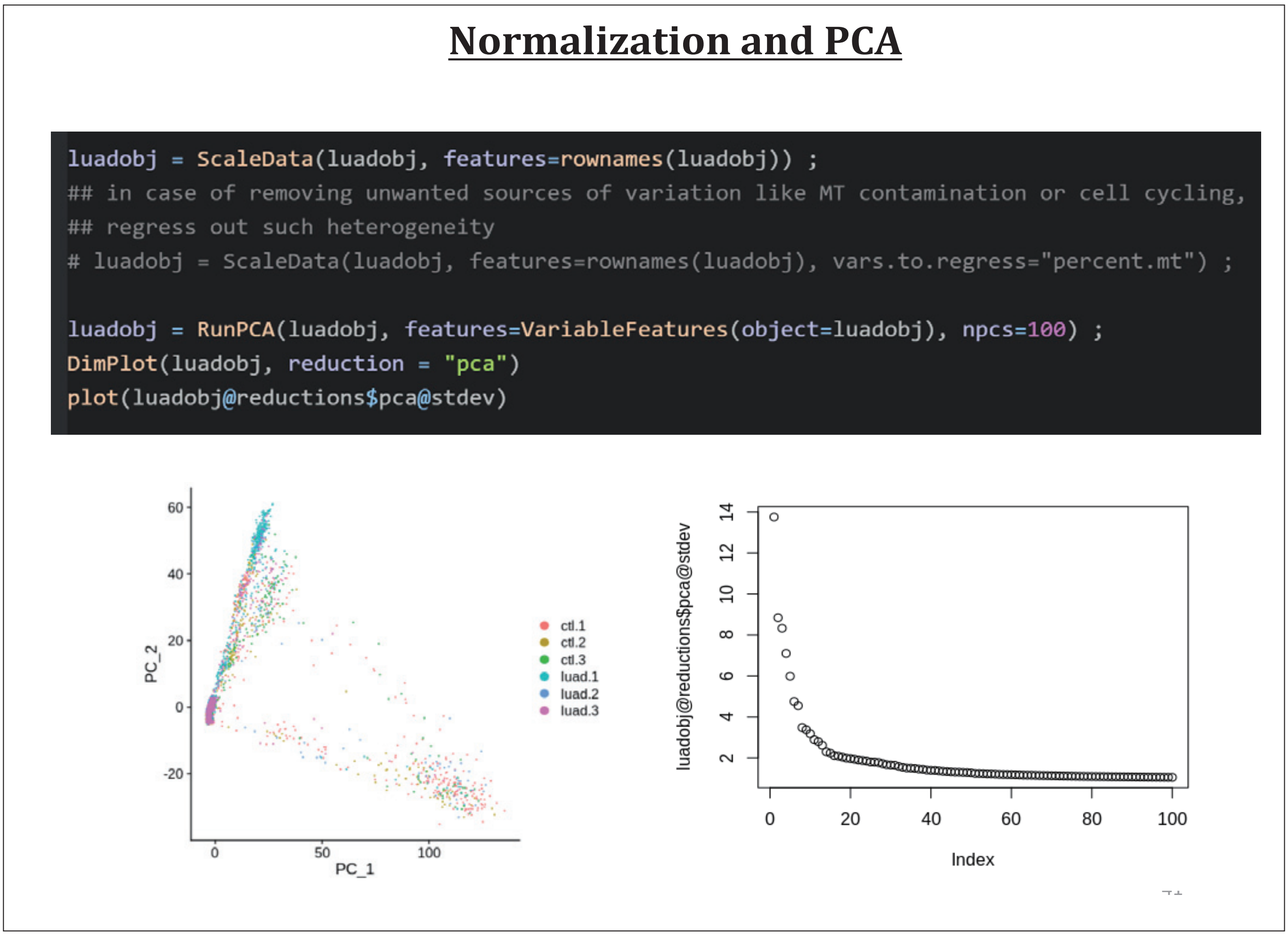
Normalization



```
# Identify the 10 most highly variable genes
top10 <- head(VariableFeatures(luadobj), 10)

# plot variable features with and without labels
variable_plot <- VariableFeaturePlot(luadobj)
variable_plot.wTop10 <- LabelPoints(plot = variable_plot, points = top10, repel = TRUE)
variable_plot.wTop10
```

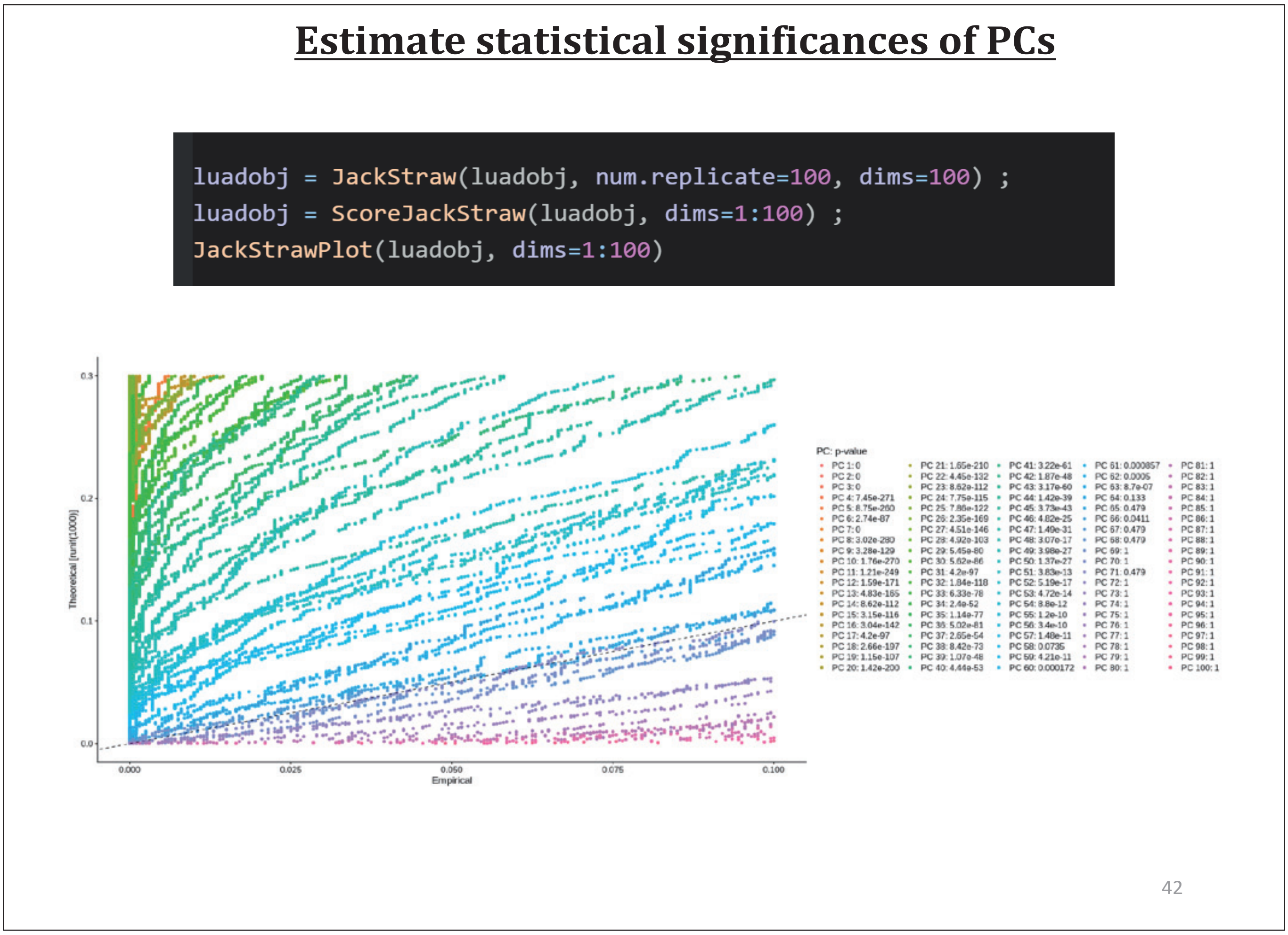




Normalization and PCA

```
luadobj = ScaleData(luadobj, features=rownames(luadobj)) ;
## in case of removing unwanted sources of variation like MT contamination or cell cycling,
## regress out such heterogeneity
# luadobj = ScaleData(luadobj, features=rownames(luadobj), vars.to.regress="percent.mt") ;

luadobj = RunPCA(luadobj, features=VariableFeatures(object=luadobj), npcs=100) ;
DimPlot(luadobj, reduction = "pca")
plot(luadobj@reductions$pca@stdev)
```



Estimate statistical significances of PCs

```
luadobj = JackStraw(luadobj, num.replicate=100, dims=100) ;
luadobj = ScoreJackStraw(luadobj, dims=1:100) ;
JackStrawPlot(luadobj, dims=1:100)
```

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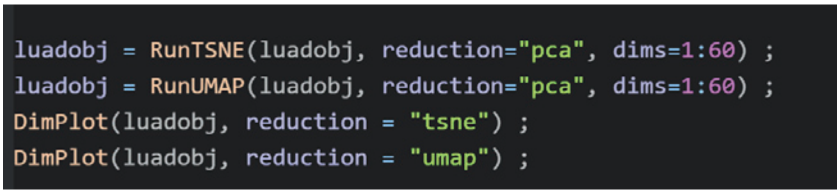
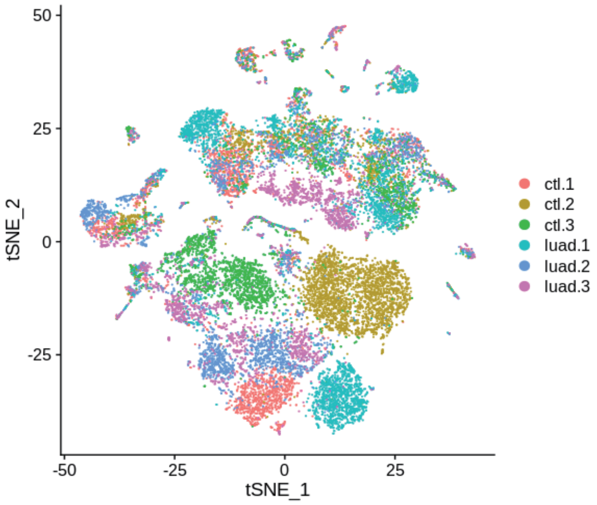
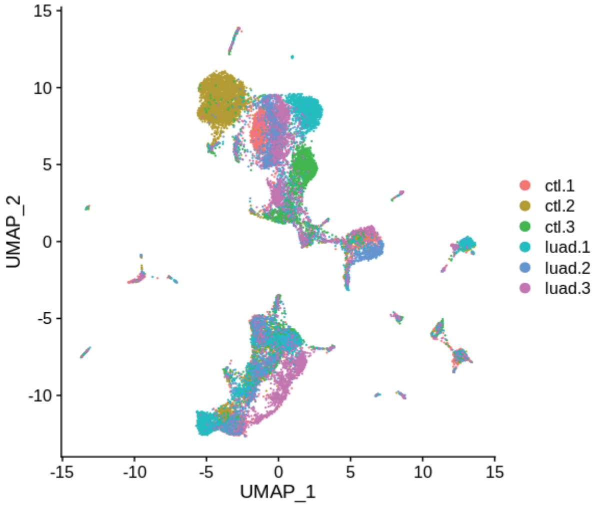
Dimensional reduction and visualization

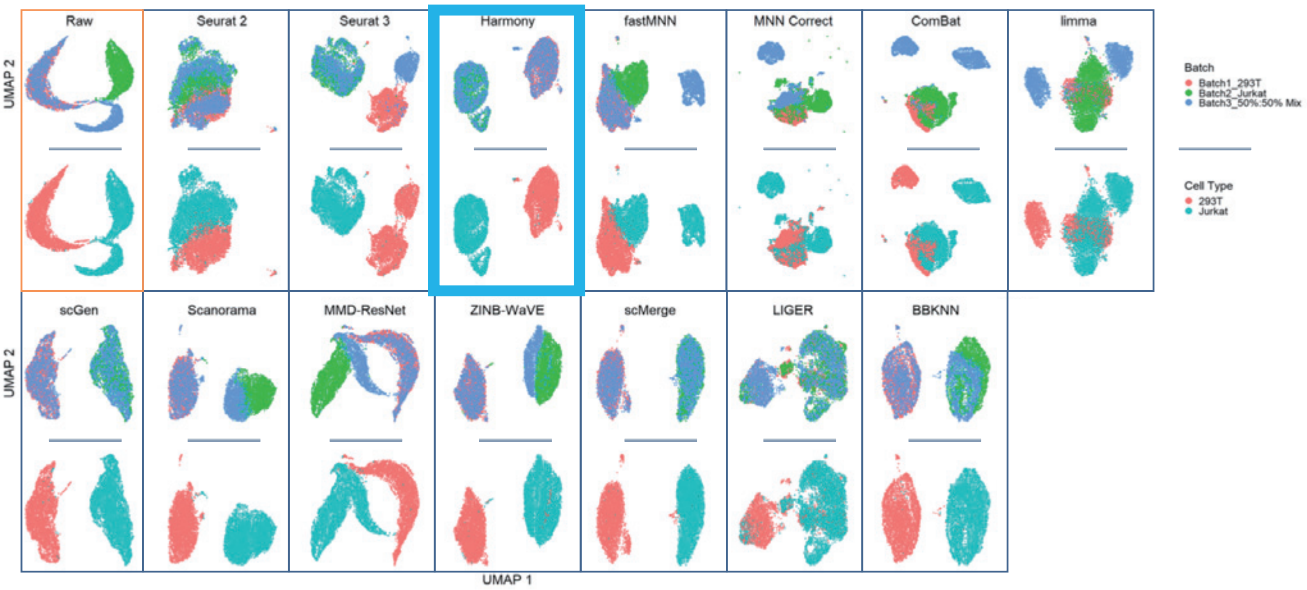


```
luadobj = RunTSNE(luadobj, reduction="pca", dims=1:60) ;
luadobj = RunUMAP(luadobj, reduction="pca", dims=1:60) ;
DimPlot(luadobj, reduction = "tsne") ;
DimPlot(luadobj, reduction = "umap") ;
```

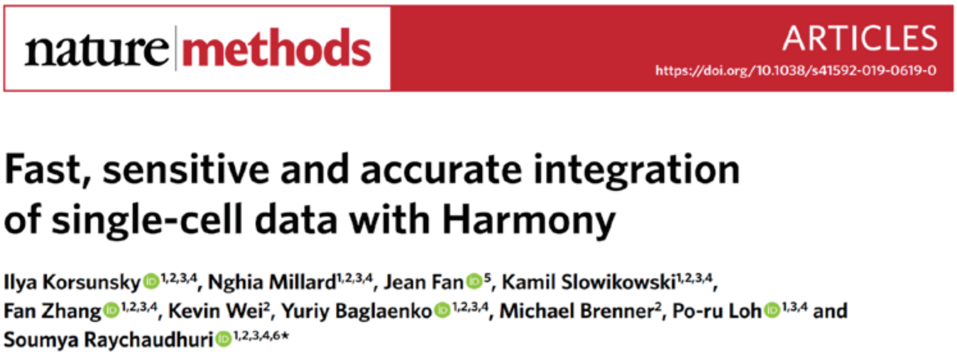




Batch-correction



Batch-correction

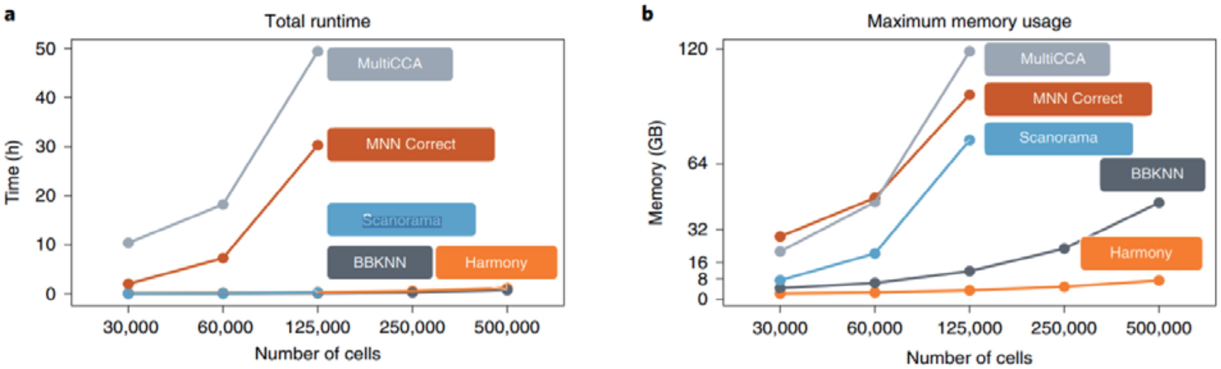


nature methods ARTICLES

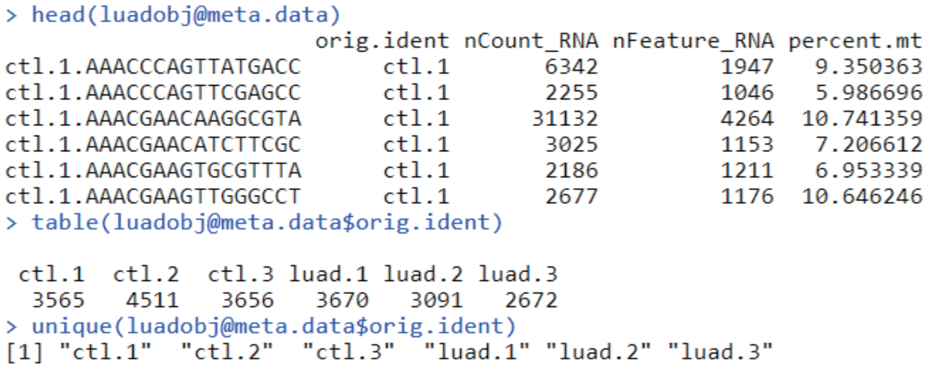
https://doi.org/10.1038/s41592-019-0619-0

Fast, sensitive and accurate integration of single-cell data with Harmony

Ilya Korsunsky[1,2,3,4], Nghia Millard[1,2,3,4], Jean Fan[5], Kamil Slowikowski[1,2,3,4], Fan Zhang[1,2,3,4], Kevin Wei[2], Yuriy Baglaenko[1,2,3,4], Michael Brenner[2], Po-ru Loh[1,3,4] and Soumya Raychaudhuri[1,2,3,4,6*]

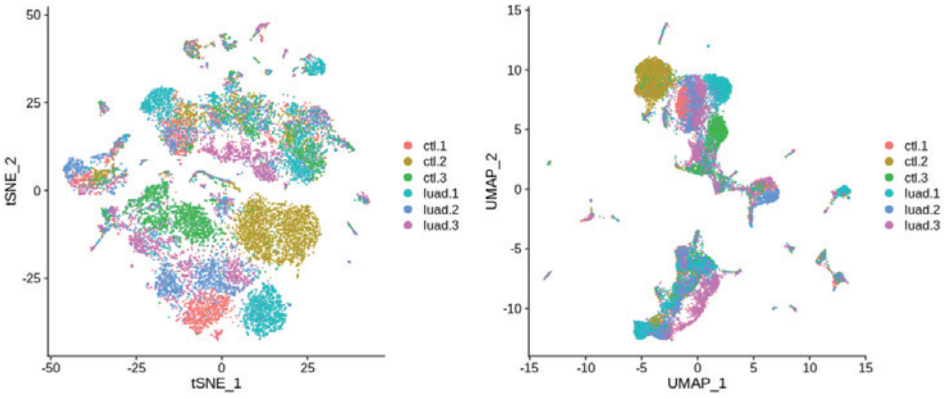


Batch-correction

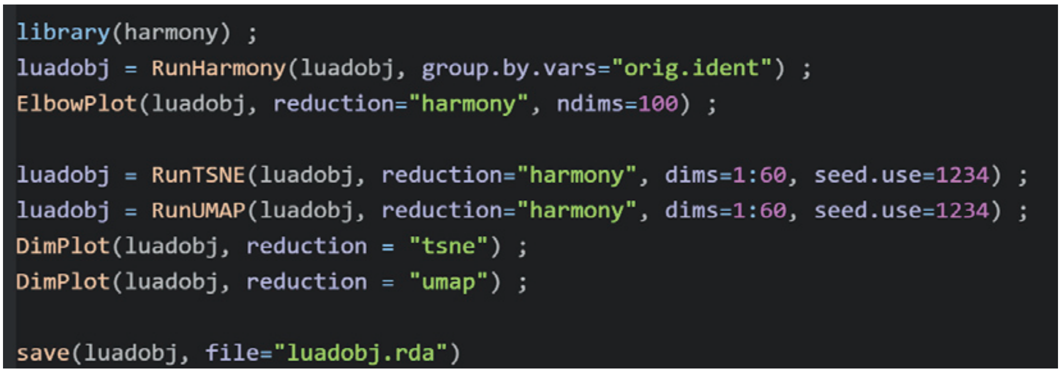


```
> head(luadobj@meta.data)
                        orig.ident nCount_RNA nFeature_RNA percent.mt
ctl.1.AAACCCAGTTATGACC       ctl.1       6342         1947   9.350363
ctl.1.AAACCCAGTTCGAGCC       ctl.1       2255         1046   5.986696
ctl.1.AAACGAACAAGGCGTA       ctl.1      31132         4264  10.741359
ctl.1.AAACGAACATCTTCGC       ctl.1       3025         1153   7.206612
ctl.1.AAACGAAGTGCGTTTA       ctl.1       2186         1211   6.953339
ctl.1.AAACGAAGTTGGGCCT       ctl.1       2677         1176  10.646246
> table(luadobj@meta.data$orig.ident)

 ctl.1  ctl.2  ctl.3 luad.1 luad.2 luad.3 
  3565   4511   3656   3670   3091   2672 
> unique(luadobj@meta.data$orig.ident)
[1] "ctl.1"  "ctl.2"  "ctl.3"  "luad.1" "luad.2" "luad.3"
```



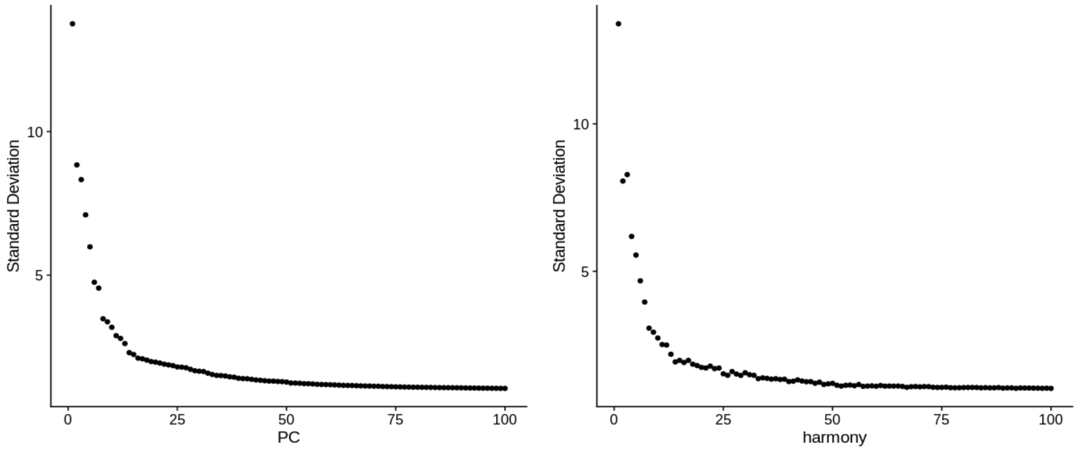
Batch-correction



```
library(harmony) ;
luadobj = RunHarmony(luadobj, group.by.vars="orig.ident") ;
ElbowPlot(luadobj, reduction="harmony", ndims=100) ;

luadobj = RunTSNE(luadobj, reduction="harmony", dims=1:60, seed.use=1234) ;
luadobj = RunUMAP(luadobj, reduction="harmony", dims=1:60, seed.use=1234) ;
DimPlot(luadobj, reduction = "tsne") ;
DimPlot(luadobj, reduction = "umap") ;

save(luadobj, file="luadobj.rda")
```



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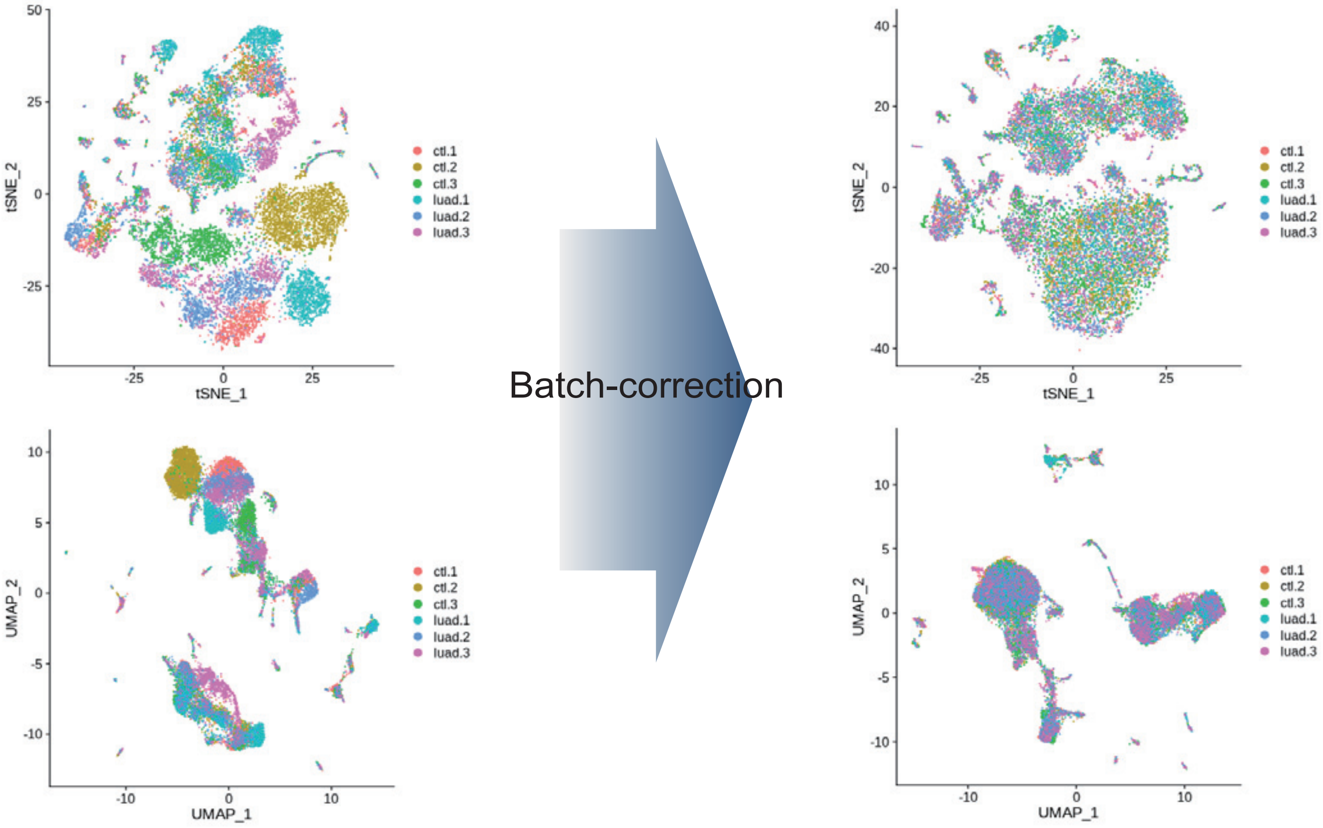
Batch-correction



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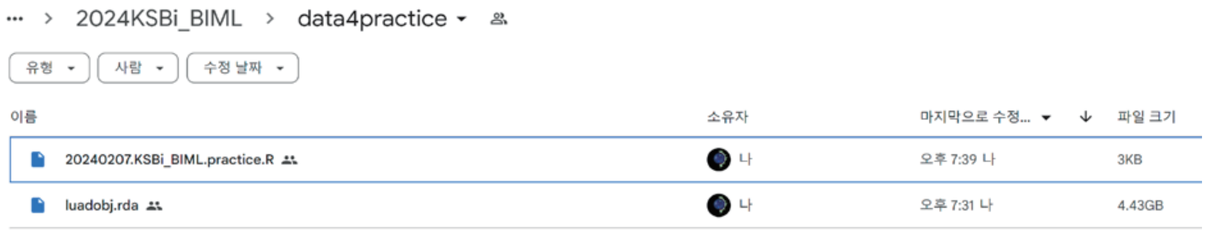
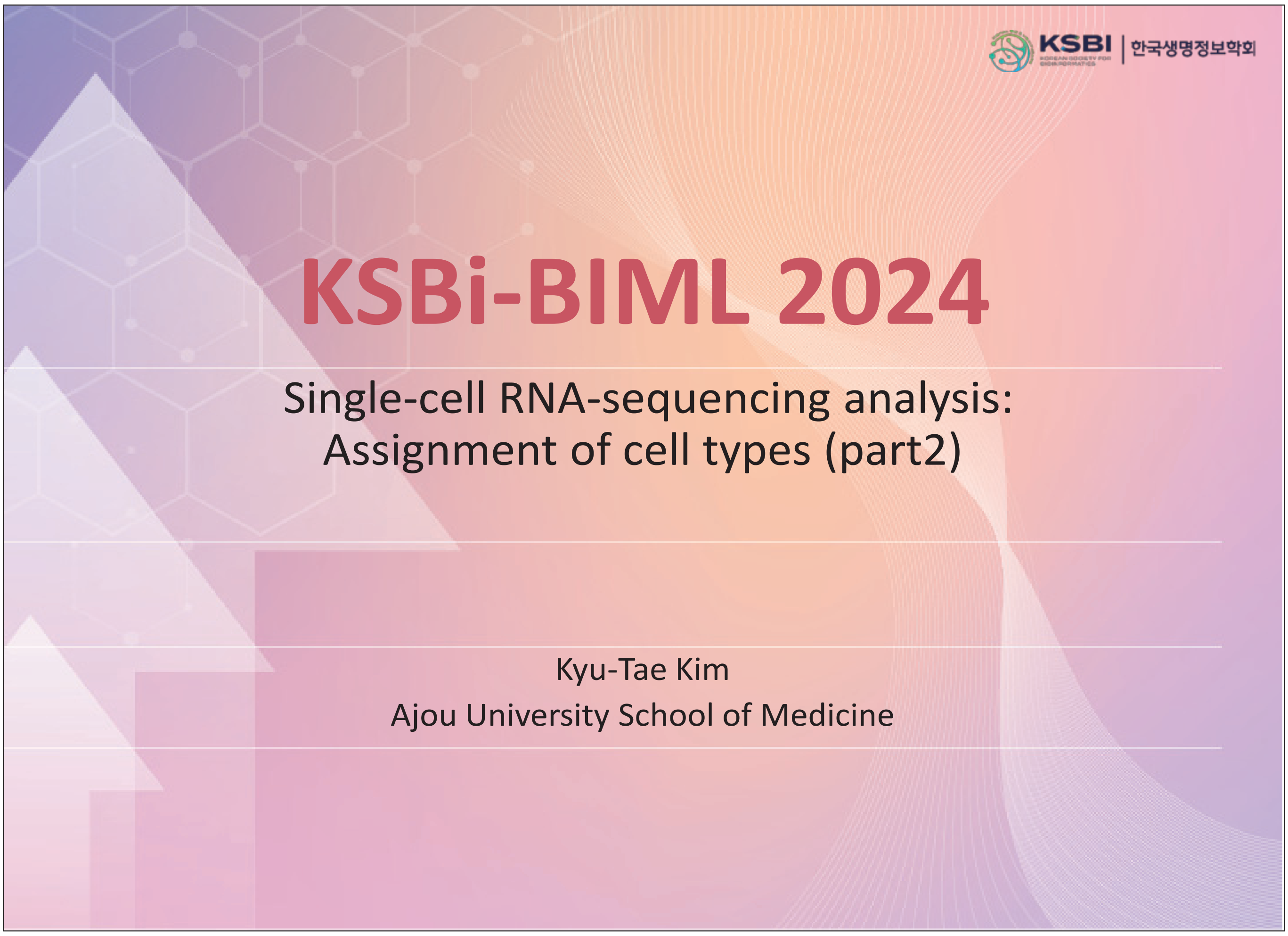
https://drive.google.com/drive/folders/1R-5vQUaDBk59n-Iu5f635ANcHhBqw57j?usp=sharing



이름	소유자	마지막으로 수정... ↓	파일 크기
20240207.KSBi_BIML.practice.R	나	오후 7:39 나	3KB
luadobj.rda	나	오후 7:31 나	4.43GB

Thank you!

KIMQTAE@ajou.ac.kr



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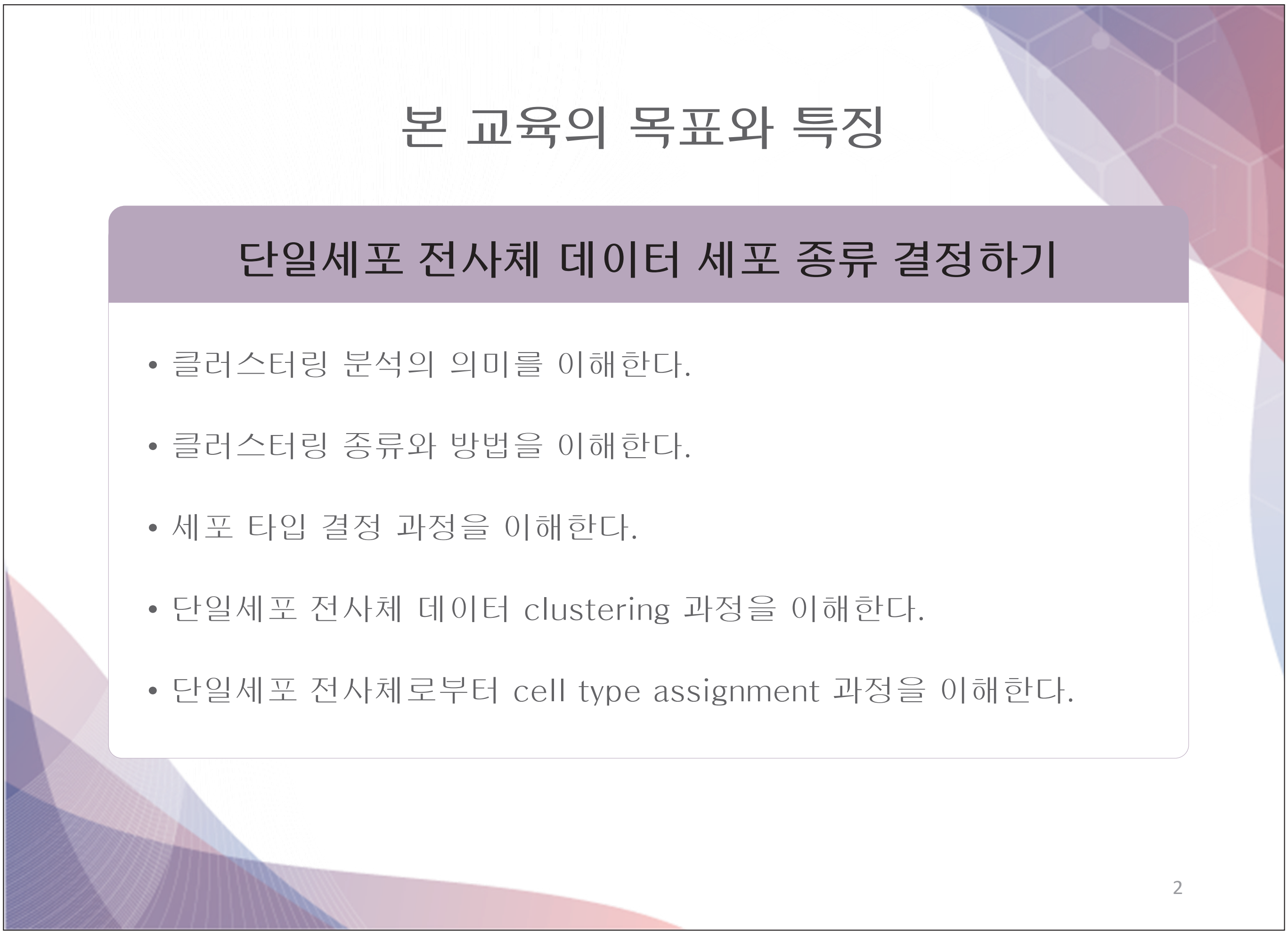
KSBi-BIML 2024

Single-cell RNA-sequencing analysis: Assignment of cell types (part2)

Kyu-Tae Kim
Ajou University School of Medicine

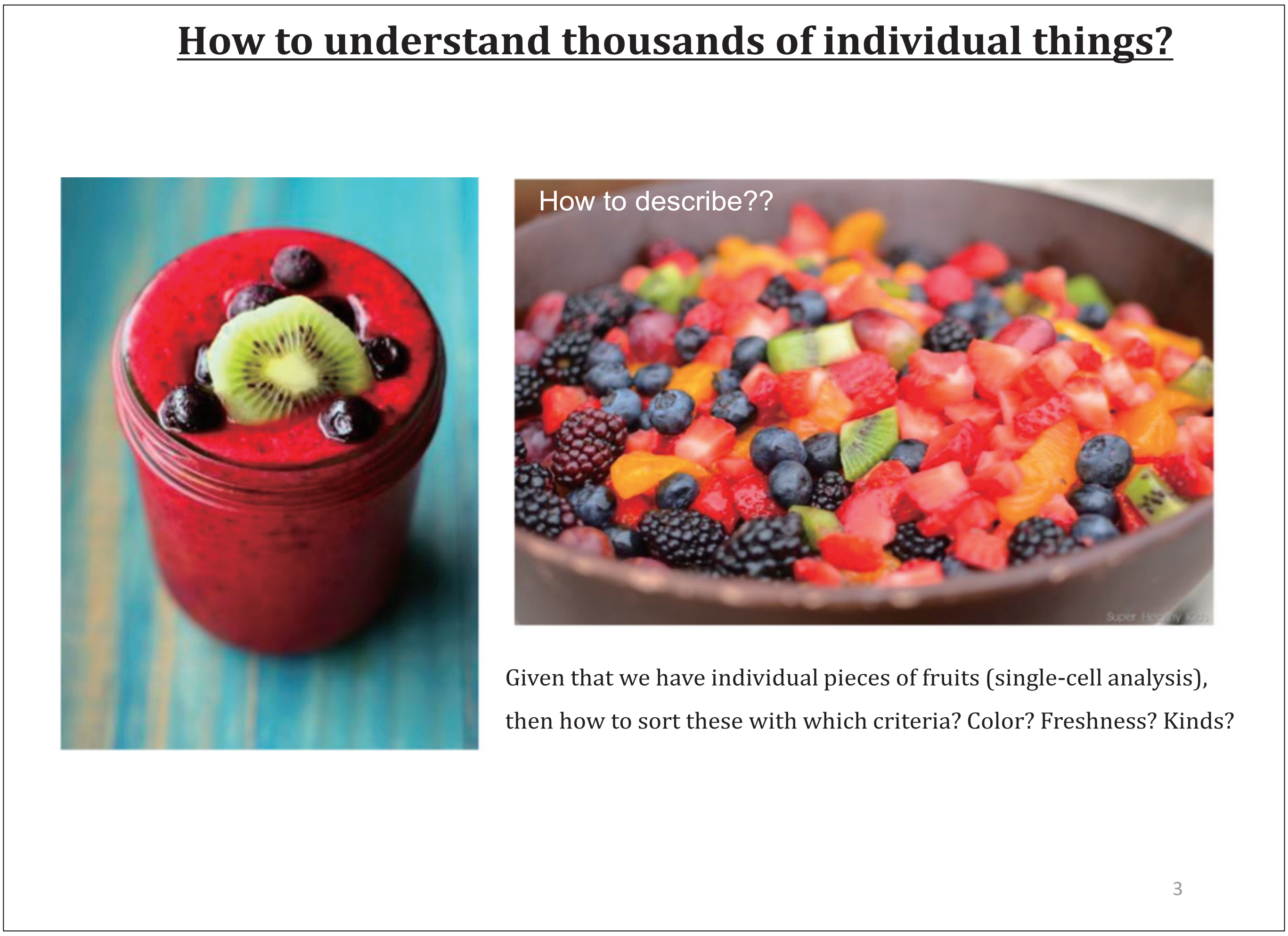


본 교육의 목표와 특징

단일세포 전사체 데이터 세포 종류 결정하기

- 클러스터링 분석의 의미를 이해한다.
- 클러스터링 종류와 방법을 이해한다.
- 세포 타입 결정 과정을 이해한다.
- 단일세포 전사체 데이터 clustering 과정을 이해한다.
- 단일세포 전사체로부터 cell type assignment 과정을 이해한다.

2

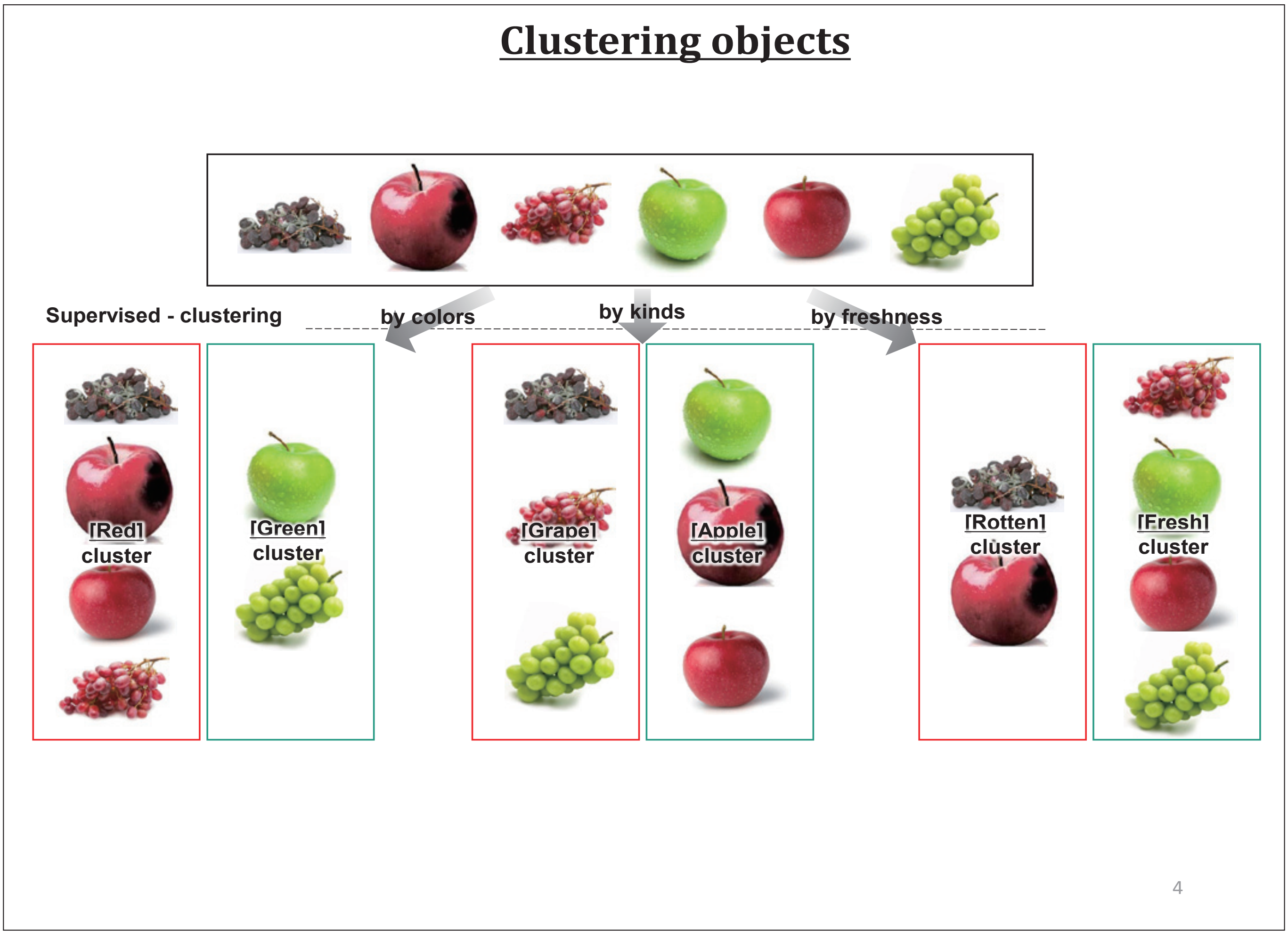


How to understand thousands of individual things?

How to describe??

Given that we have individual pieces of fruits (single-cell analysis), then how to sort these with which criteria? Color? Freshness? Kinds?

3



Clustering objects

Supervised - clustering

by colors

by kinds

by freshness

4

Supervised vs. Un-supervised clustering

Supervised clustering

> The classes are predefined, and the task is to understand the basis for the classification from a set of labeled objects (training or learning set).

> This information is then used to classify future observations.

- Discriminant analysis
- Class prediction
- Supervised pattern recognition

Un-supervised clustering

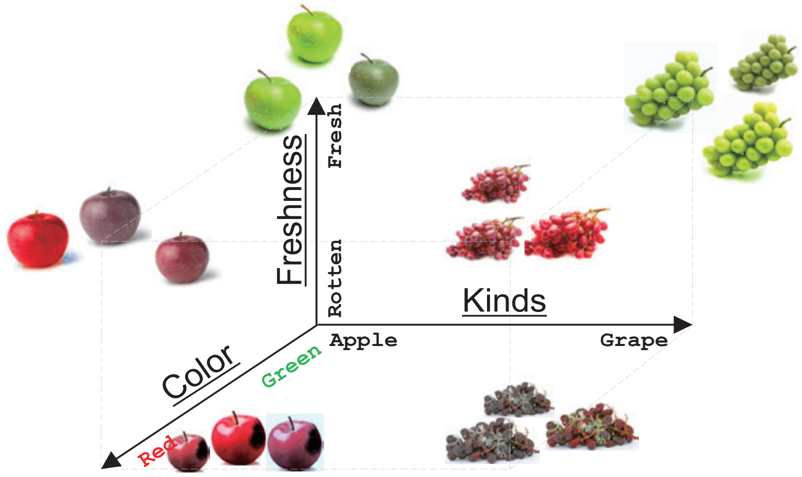
> The classes are unknown a priori and need to be "discovered" from the data.

- Cluster analysis
- Class discovery
- Unsupervised pattern recognition

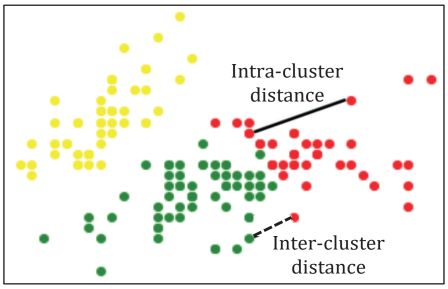
Clustering analysis

> **Finding groups** of objects such that the objects in a group will be similar (or related) to one another and different from (or unrelated to) the objects in other groups

> **Cluster**: a collection of 'similar' data



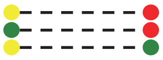
Evaluation of clustering



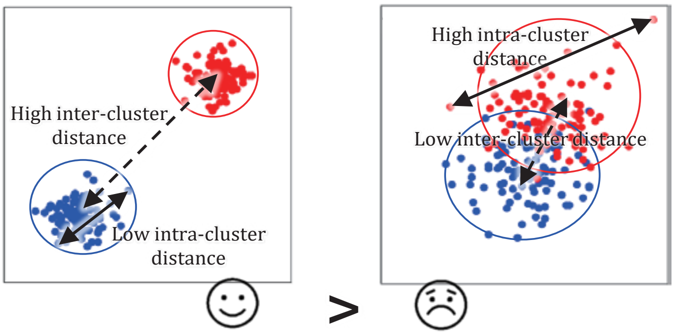
> Intra-cluster distance:

the distance among members of a cluster

> Inter-cluster distance:



the distance between two different clusters


High inter-cluster distance
Low intra-cluster distance
>
High intra-cluster distance
Low inter-cluster distance

> A **good clustering** method will produce high quality clusters with

Low intra-class distance = High intra-class similarity

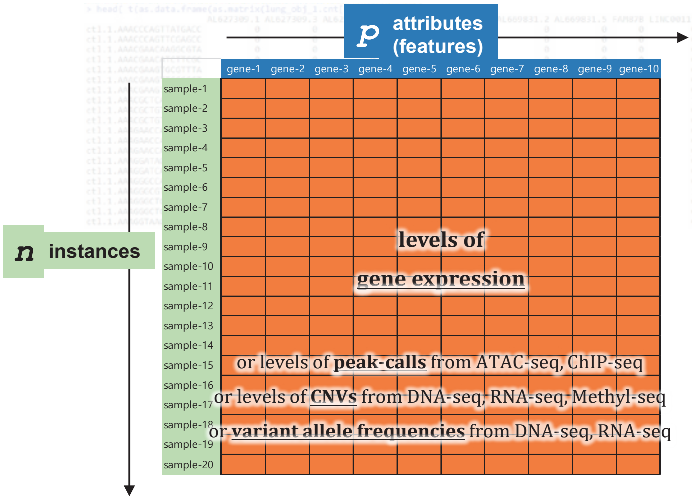
High inter-class distance = Low inter-class similarity

> How to determine ‘**similarity**’?

> How to measure ‘**distance**’?

클러스터링 종류와 방법

Similarity measures with a gene expression table



p attributes (features)

n instances

gene-1 gene-2 gene-3 gene-4 gene-5 gene-6 gene-7 gene-8 gene-9 gene-10

sample-1 … sample-20

levels of gene expression

or levels of **peak-calls** from ATAC-seq, ChIP-seq

or levels of **CNVs** from DNA-seq, RNA-seq, Methyl-seq

or **variant allele frequencies** from DNA-seq, RNA-seq

Data matrix

> *n*: size of the data (how many samples)

> *p*: attributes of the data (how many genes)

$$\begin{bmatrix} x_{11} & \cdots & x_{1f} & \cdots & x_{1p} \\ \vdots & \cdots & \vdots & \cdots & \vdots \\ x_{i1} & \cdots & x_{if} & \cdots & x_{ip} \\ \vdots & \cdots & \vdots & \cdots & \vdots \\ x_{n1} & \cdots & x_{nf} & \cdots & x_{np} \end{bmatrix}$$

Dimensionality

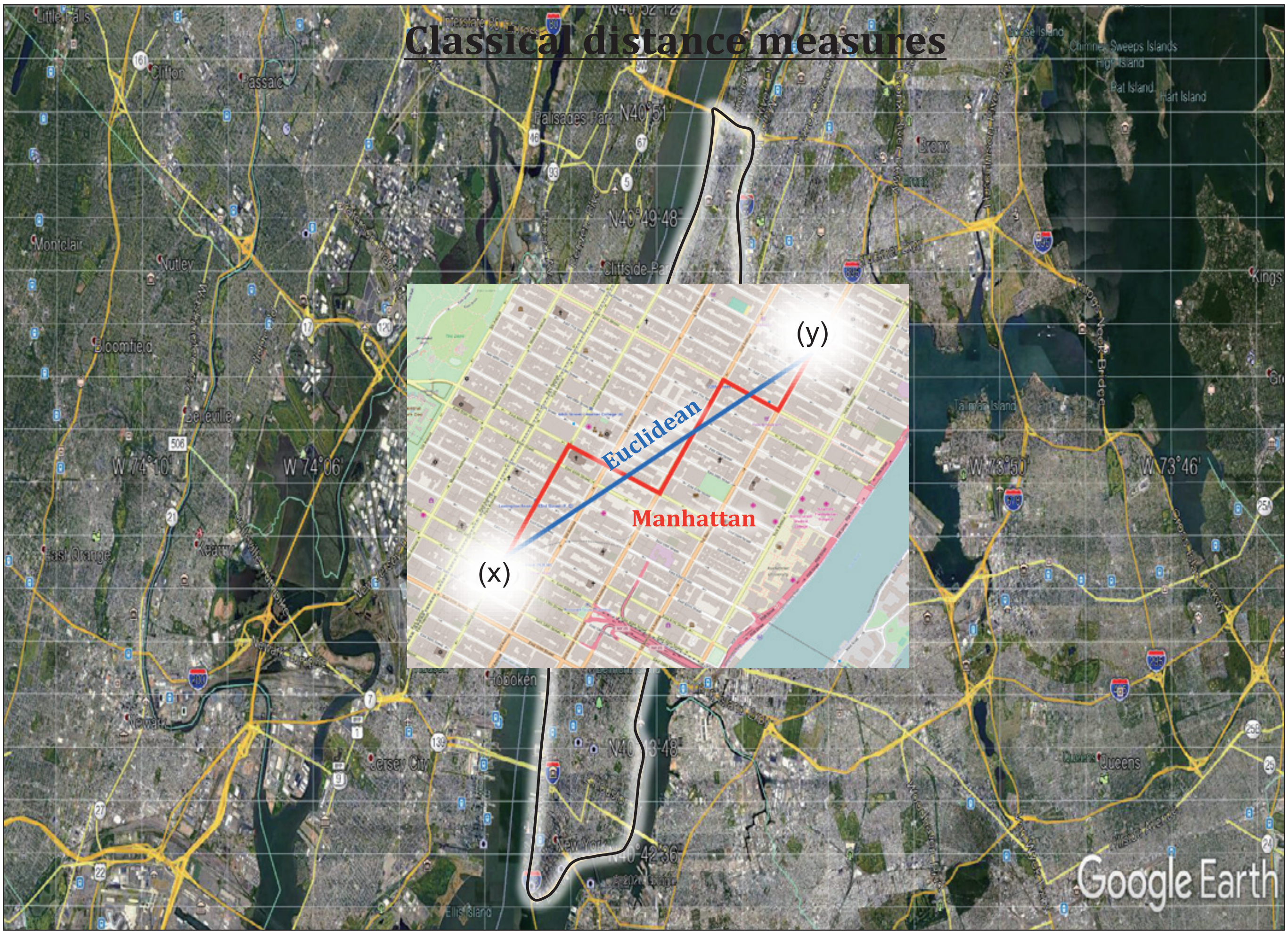
= ***n*** x ***p***

Distance matrix

(dissimilarity matrix)

$$\begin{bmatrix} 0 & & & & \\ d(2,1) & 0 & & & \\ d(3,1) & d(3,2) & 0 & & \\ \vdots & \vdots & \vdots & & \\ d(n,1) & d(n,1) & \cdots & \cdots & 0 \end{bmatrix}$$

Classical distance measures



Measures of relative distances

> Pearson correlation

- Measuring the degree of a linear relationship between two profiles

$$d_{cor}(x,y) = 1 - \frac{\sum_{i=1}^{n}(x_i - \bar{x})(y_i - \bar{y})}{\sqrt{\sum_{i=1}^{n}(x_i - \bar{x})^2 \sum_{i=1}^{n}(y_i - \bar{y})^2}}$$

> Eisen cosine correlation

- A special case of Pearson's correlation with ***x*** and ***y*** both replaced by zero

$$d_{eisen}(x,y) = 1 - \frac{\left|\sum_{i=1}^{n} x_i y_i\right|}{\sqrt{\sum_{i=1}^{n} x_i^2 \sum_{i=1}^{n} y_i^2}}$$

Parametric

> Spearman correlation

- Measuring the correlation between the rank of ***x*** and the rank of ***y*** variables

$$d_{spear}(x,y) = 1 - \frac{\sum_{i=1}^{n}(x'_i - \bar{x}')(y'_i - \bar{y}')}{\sqrt{\sum_{i=1}^{n}(x'_i - \bar{x}')^2 \sum_{i=1}^{n}(y'_i - \bar{y}')^2}}$$

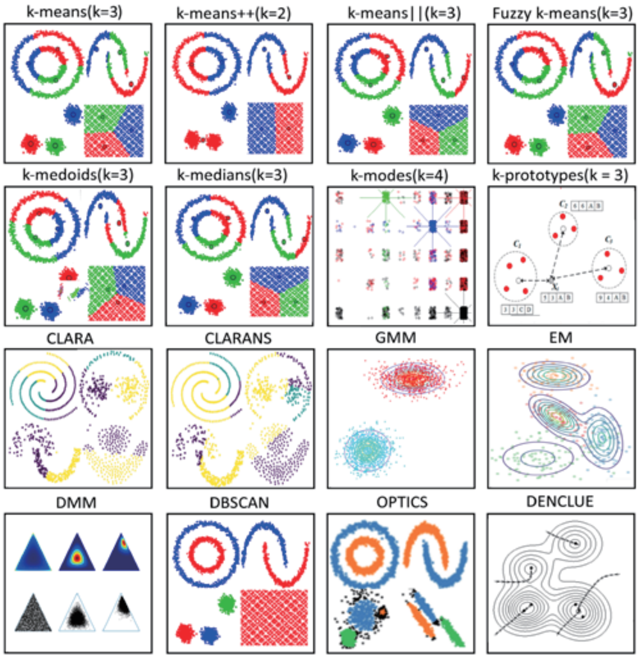
> Kendall correlation

- Measuring the correspondence between the ranking of ***x*** and ***y*** variables

$$d_{kend}(x,y) = 1 - \frac{n_c - n_d}{\frac{1}{2}n(n-1)}$$

non-Parametric

Clustering methods



> Hierarchical clustering

> Partitioning clustering

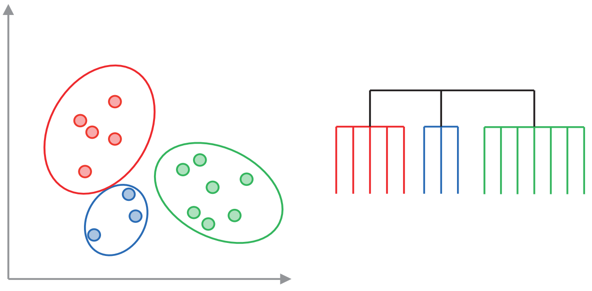
- K-medoids
- PAM (Partitioning Around Medoid)
- SOM (Self Organizing Maps)

> Advanced clustering

- Hybrid clustering methods
- Fuzzy clustering
- Model-based clustering
- Density-based clustering
- Graph-based clustering
- and ...

Hierarchical clustering

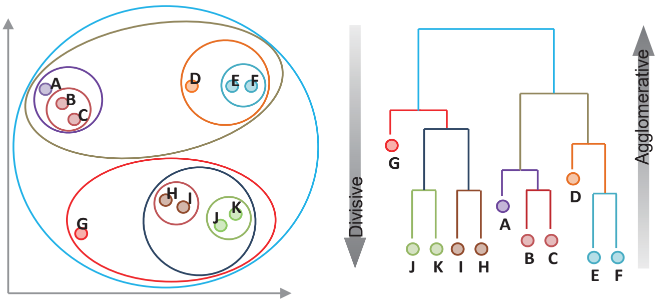
non-Hierarchical



(Nested clusters)

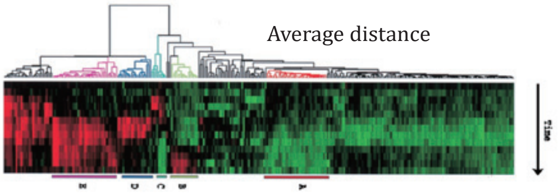
(Dendrogram)

Hierarchical

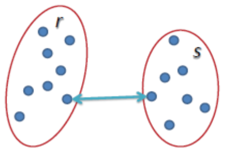


Hierarchical clustering

- Hierarchical clustering was the first algorithm used in microarray research to cluster genes. (David Bostein group, PNAS 1998)



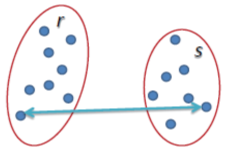
- First, each object is assigned to its own cluster. Then, iteratively, the two most similar clusters are joined, representing a new node of the clustering tree. The similarity matrix is updated. This process is repeated until only a single cluster remains. (agglomerative clustering)



$$L(r,s)=\min(D(x_{ri},x_{sj}))$$

> Single linkage

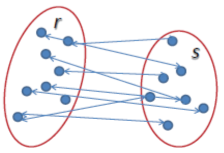
- Smallest distance



$$L(r,s)=\max(D(x_{ri},x_{sj}))$$

> Complete linkage

- Largest distance

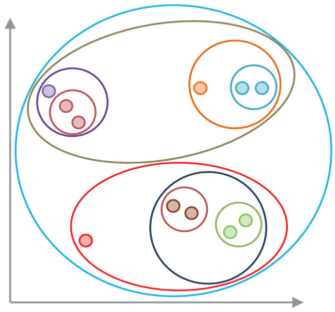


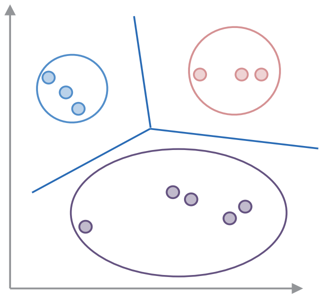
$$L(r,s)=\frac{1}{n_r n_s}\sum_{i=1}^{n_r}\sum_{j=1}^{n_s}D(x_{ri},x_{sj})$$

> Average linkage

- Average distance

Partitioning clustering





> Hierarchical

- Clustering is hierarchical decomposition (i.e., multiple levels)
- It can not correct erroneous merges or splits

> Partitioning

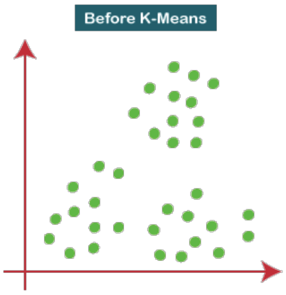
- It find mutually exclusive clusters of spherical shape
- It may use mean or medoid to represent cluster center
- It may effective for small- to medium-size data sets

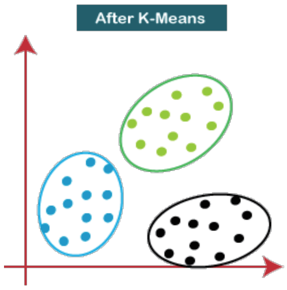
K-means clustering

- Number of cluster, ***K***, must be specified
- Each cluster is associated with an averaged point (centroid)
- Each point is assigned to the cluster with the closest centroid

- Basic algorithm

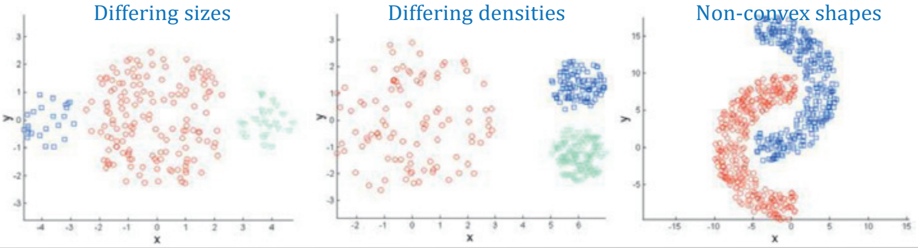
```
1: Select K points as the initial centroids.
2: repeat
3:     From K clusters by assigning all points to the closest centroid.
4:     Recompute the centroid of each cluster.
5: until The centroids does not change
```





Limitation of *K-means* clustering

- Applicable only when mean is defined, then what about categorical data?
- Need to specify *K*, the number of clusters, in advance
- Unable to handle noisy data and outliers
- Not suitable to discover clusters with



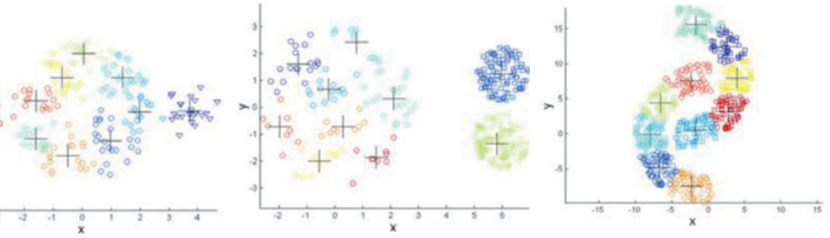
Differing sizes

Differing densities

Non-convex shapes

Overcoming limitations

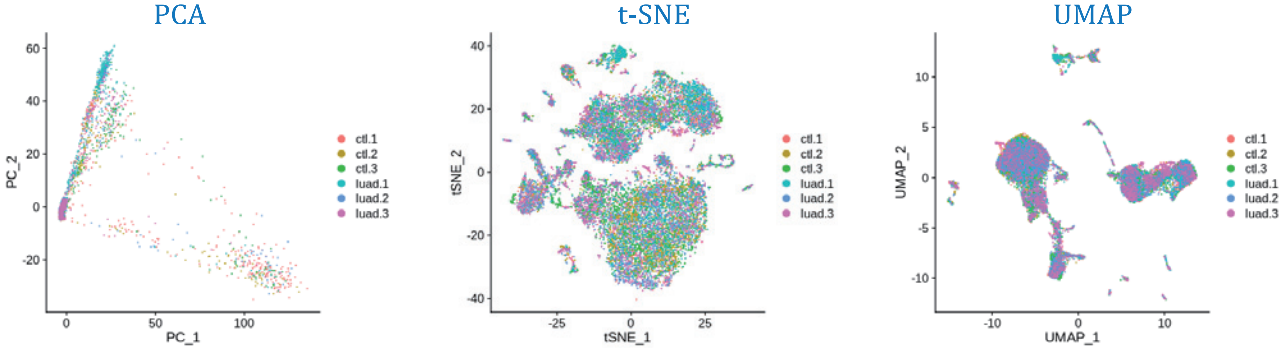
- Using many clusters (i.e., high *K*)
- Using K-medoids, instead of k-means which is sensitive to outliers



Dimensional reduction for visualization

> Projection methods

- PCA (Principal Component Analysis)
- t-SNE (t-distributed Stochastic Neighbor Embedding)
- UMAP (Uniform Manifold Approximation and Projection)

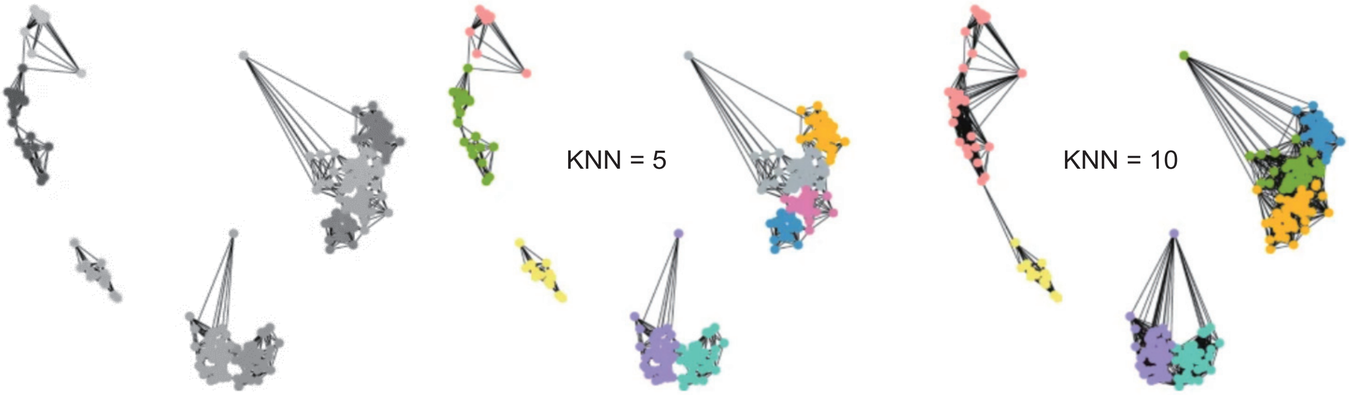


PCA

t-SNE

UMAP

Graph-based clustering



- Louvain community detection is applied to a shared-nearest-neighbor graph connecting the cells and finds tightly connected communities in the graph
- Increasing the number of neighbors when constructing the cell–cell graph indirectly decreases the resolution of graph-based clustering.

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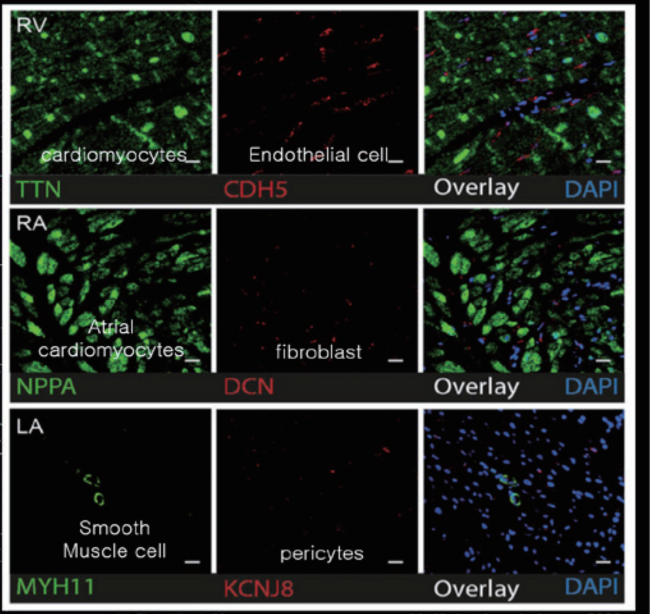
세포 유형 결정

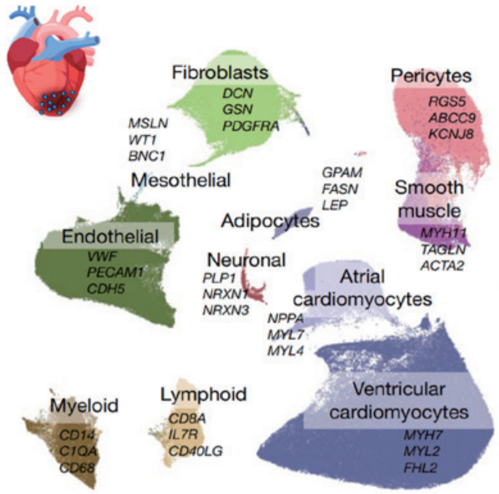
20

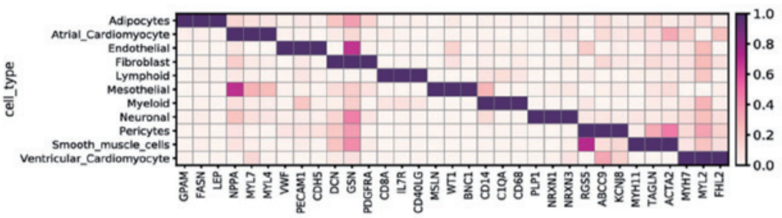
Cell-type assignment



Litviňuková et al., *Nature* 2020

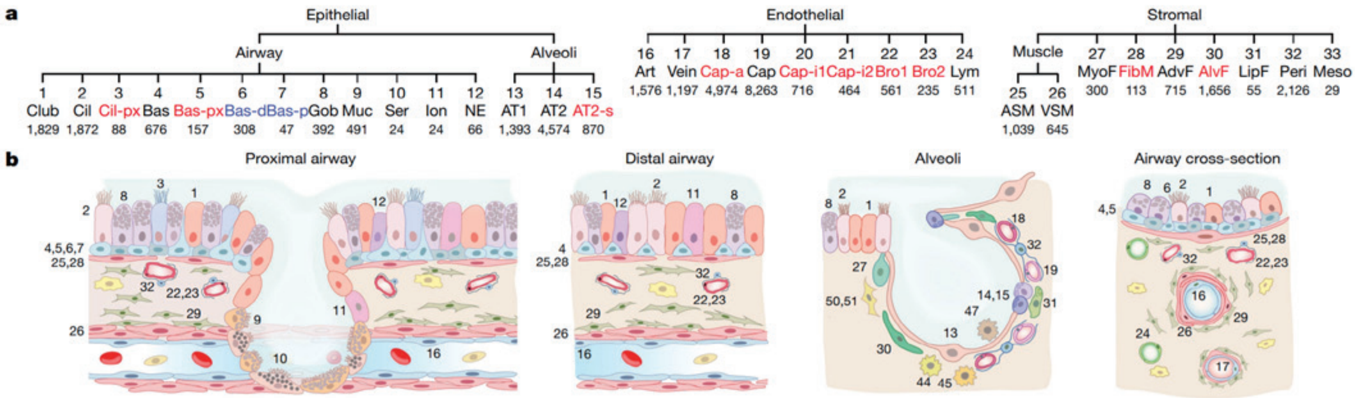
Cell-type assignment





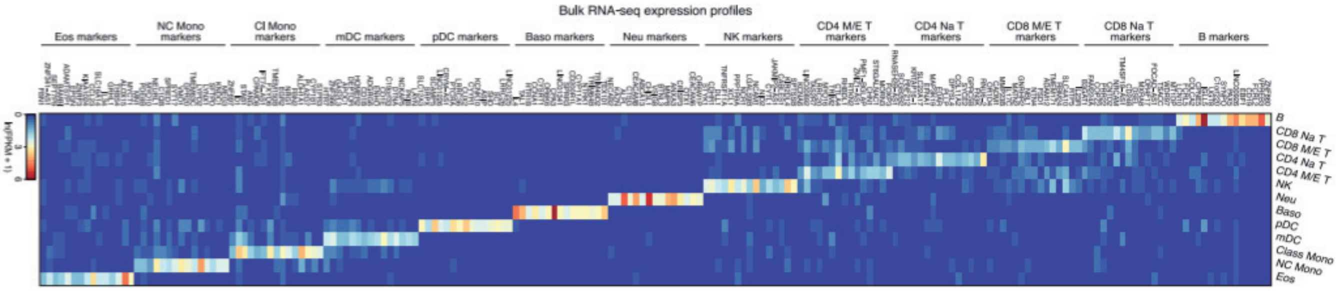
Litviňuková et al., *Nature* 2020

Cell-type assignment



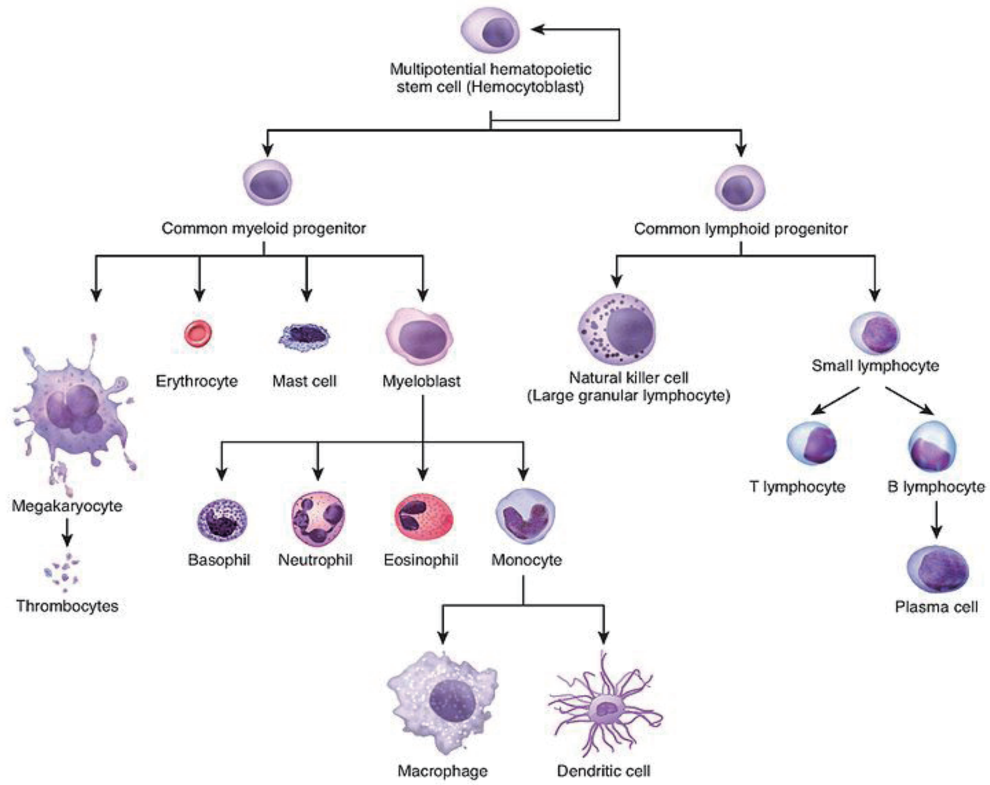
Travaglini et al., *Nature* 2020

Cell-type assignment



Travaglini et al., *Nature* 2020

Hematopoiesis



[cell type gene expression] https://dice-database.org/

[cell type gene expression] https://xteam.xbio.top/CellMarker/

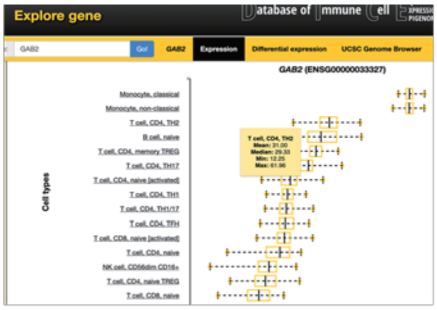
[cell type gene expression] https://panglaodb.se/

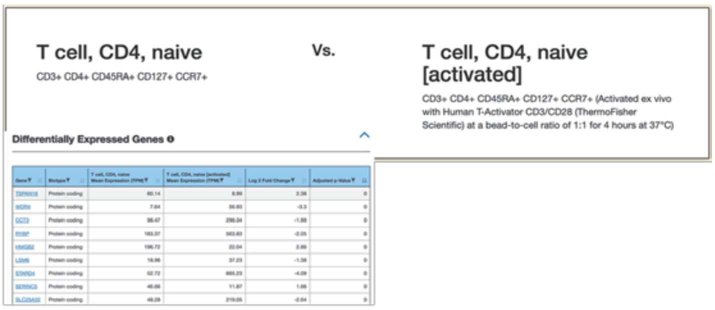
[cell type gene expression] https://cellxgene.cziscience.com/cellguide/

[cell type gene expression] https://www.celltypist.org/encyclopedia/Immune/v2

[cell types in blood/tissue marker ptn. expression] https://www.proteinatlas.org/

Useful resources to identify cell type markers





T cell, CD4, naive

CD3+ CD4+ CD45RA+ CD127+ CCR7+

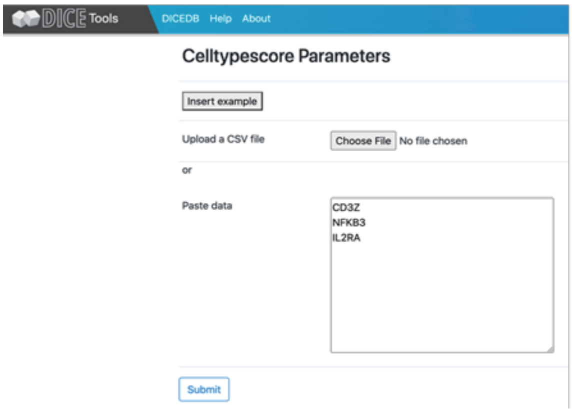
Vs.

T cell, CD4, naive [activated]

CD3+ CD4+ CD45RA+ CD127+ CCR7+ (Activated ex vivo with Human T-Activator CD3/CD28 (ThermoFisher Scientific) at a bead-to-cell ratio of 1:1 for 4 hours at 37°C)

Differentially Expressed Genes

Gene	Biotype	T cell, CD4, naive Mean Expression (TPM)	T cell, CD4, naive [activated] Mean Expression (TPM)	Log 2 Fold Change	Adjusted p-Value
TSPAN18	Protein coding	60.14	8.99	2.38	0
WDR4	Protein coding	7.64	56.93	-3.3	0
CCT3	Protein coding	58.47	296.34	-1.89	0
RYBP	Protein coding	163.37	583.83	-2.05	0
HMGB2	Protein coding	196.72	22.04	2.86	0
LSM6	Protein coding	18.96	37.23	-1.38	0
STARD4	Protein coding	52.72	665.23	-4.39	0
SERINC5	Protein coding	46.66	11.87	1.66	0
SLC25A32	Protein coding	48.28	219.05	-2.64	0



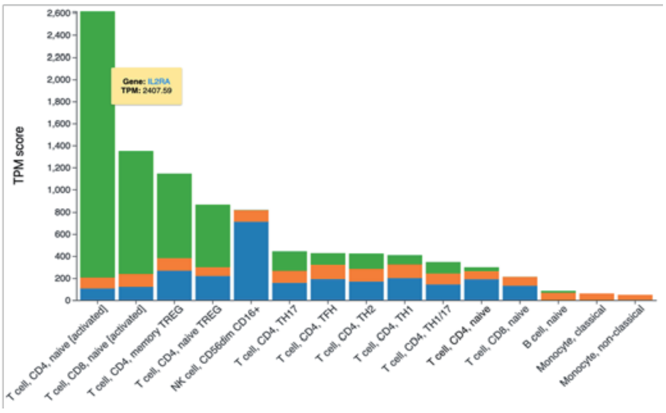
Celltypescore Parameters

Upload a CSV file

or

Paste data

CD3Z
NFKB3
IL2RA



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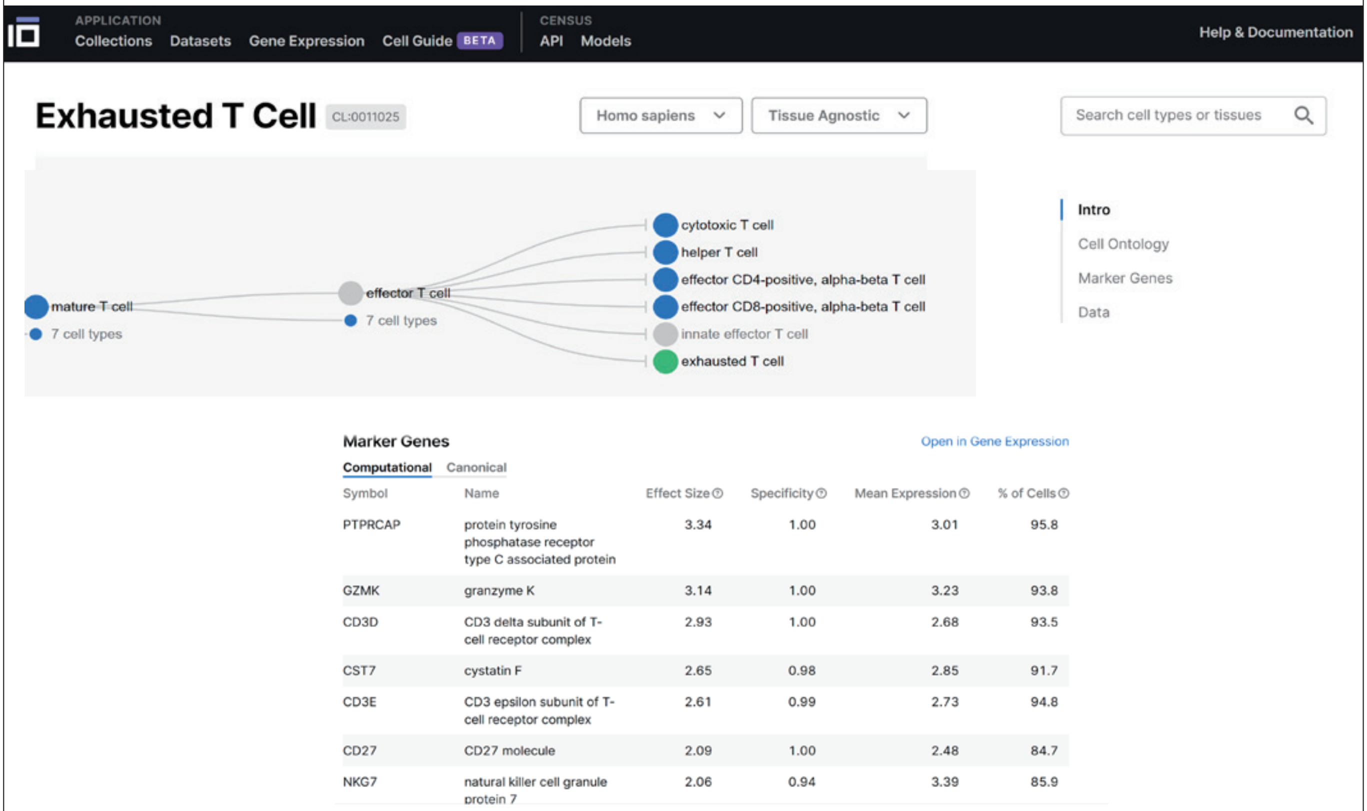
Useful resources to identify cell type markers



Exhausted T Cell CL:0011025

Marker Genes

Symbol	Name	Effect Size	Specificity	Mean Expression	% of Cells
PTPRCAP	protein tyrosine phosphatase receptor type C associated protein	3.34	1.00	3.01	95.8
GZMK	granzyme K	3.14	1.00	3.23	93.8
CD3D	CD3 delta subunit of T-cell receptor complex	2.93	1.00	2.68	93.5
CST7	cystatin F	2.65	0.98	2.85	91.7
CD3E	CD3 epsilon subunit of T-cell receptor complex	2.61	0.99	2.73	94.8
CD27	CD27 molecule	2.09	1.00	2.48	84.7
NKG7	natural killer cell granule protein 7	2.06	0.94	3.39	85.9

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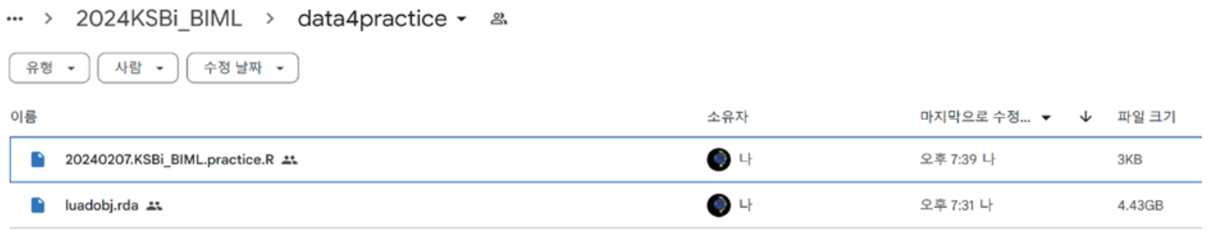
Data loading (practice)

https://drive.google.com/drive/folders/1R-5vQUaDBk59n-Iu5f635ANcHhBqw57j?usp=sharing



··· › 2024KSBi_BIML › data4practice ▾

유형 ▾ 사람 ▾ 수정 날짜 ▾

이름	소유자	마지막으로 수정... ▾ ↓	파일 크기
20240207.KSBi_BIML.practice.R	나	오후 7:39 나	3KB
luadobj.rda	나	오후 7:31 나	4.43GB

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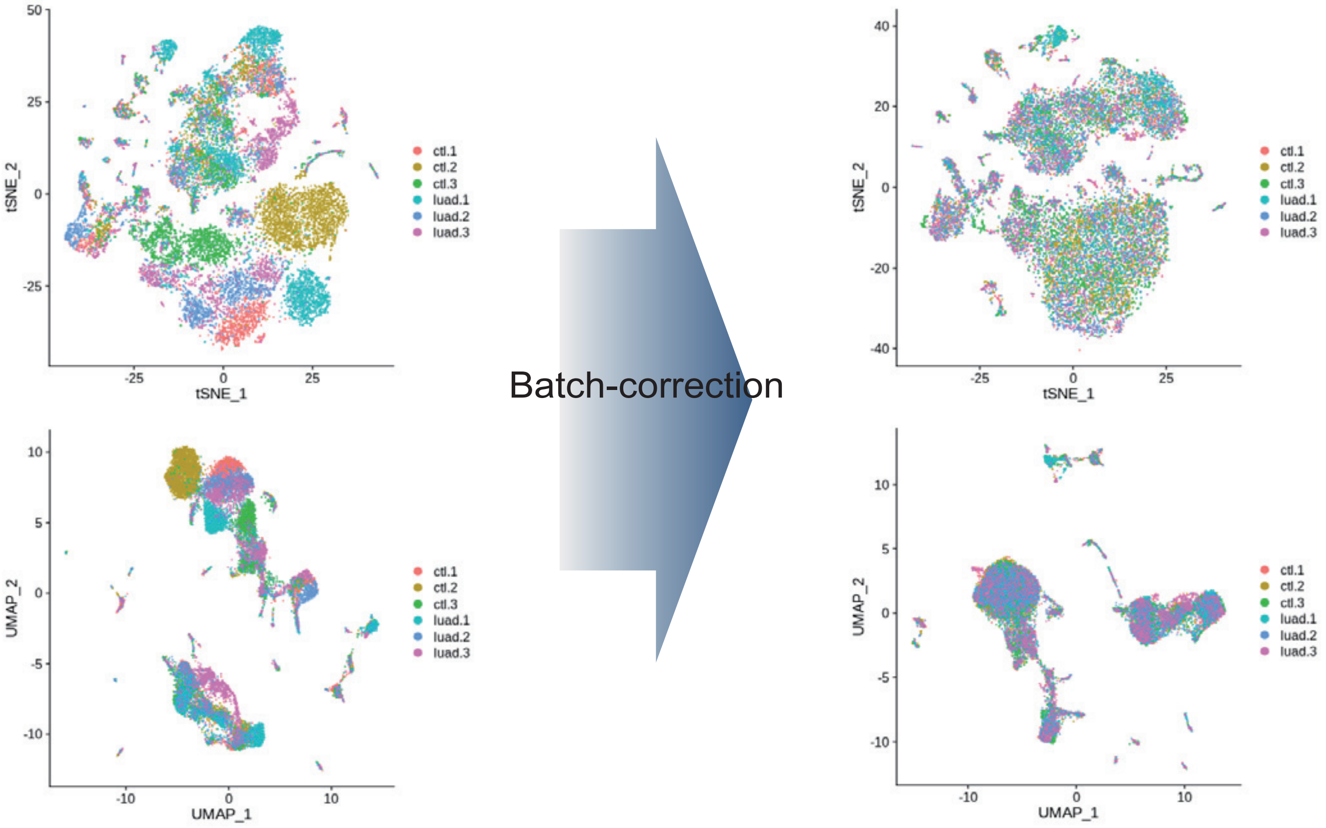
Batch-correction



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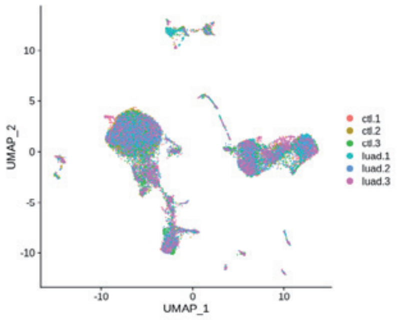
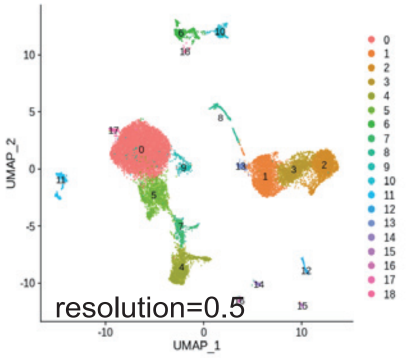
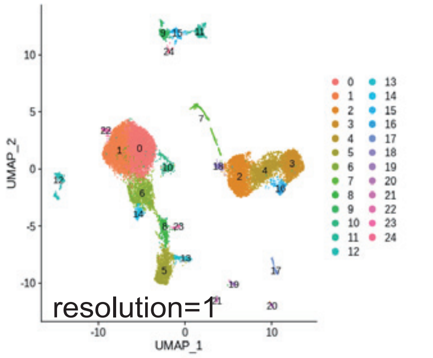
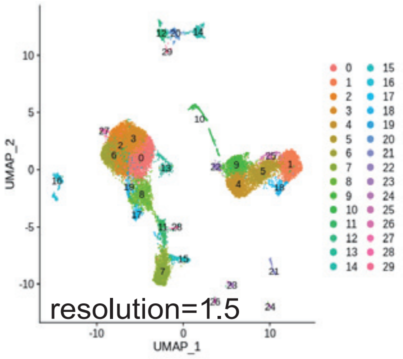
Clustering



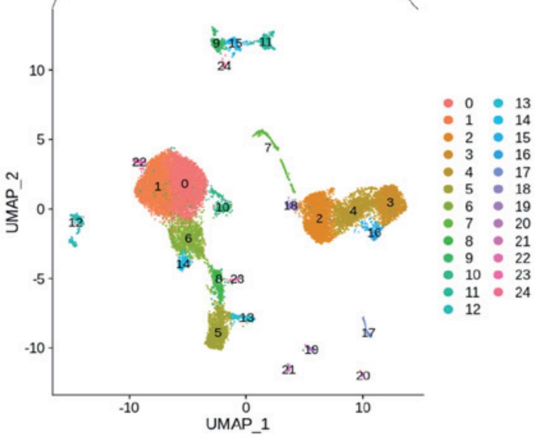
```
> luadobj = FindNeighbors(luadobj, reduction="harmony", k.param=20, dims=1:60)
Computing nearest neighbor graph
Computing SNN
> luadobj = FindClusters(luadobj, resolution=1) ;
Modularity Optimizer version 1.3.0 by Ludo Waltman and Nees Jan van Eck

Number of nodes: 21165
Number of edges: 896920

Running Louvain algorithm...
0%   10   20   30   40   50   60   70   80   90   100%
[----|----|----|----|----|----|----|----|----|----|
**************************************************|
Maximum modularity in 10 random starts: 0.8588
Number of communities: 25
Elapsed time: 3 seconds
> DimPlot(luadobj, reduction="umap", group.by="seurat_clusters", pt.size=0.001, label=T)
```



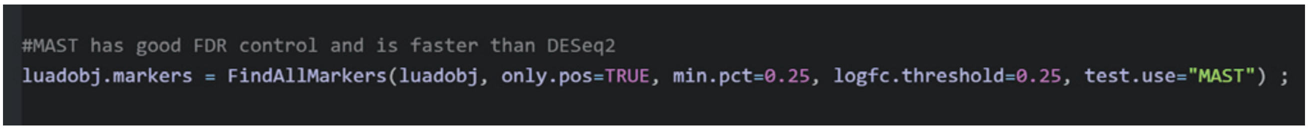




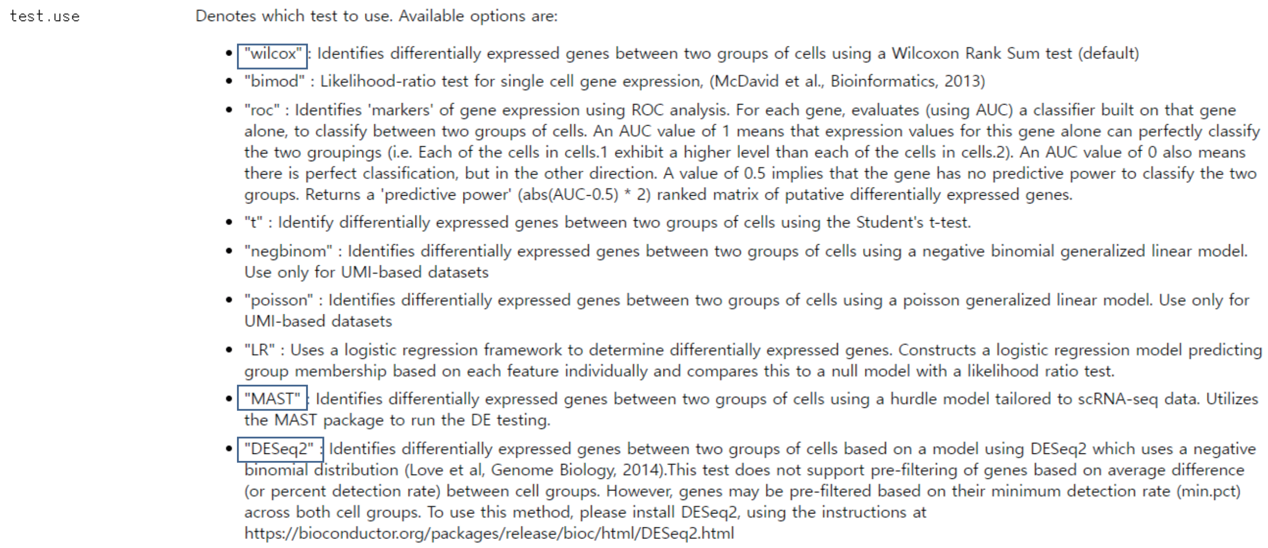
Identification of cluster-specific markers



Identification of cluster-specific markers



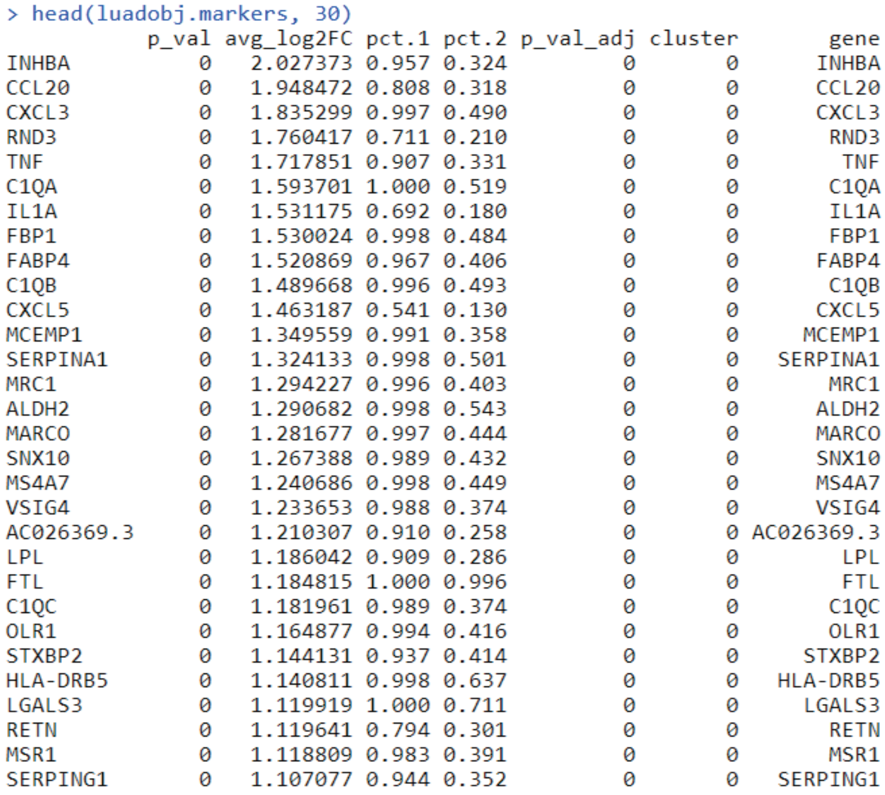
```
#MAST has good FDR control and is faster than DESeq2
luadobj.markers = FindAllMarkers(luadobj, only.pos=TRUE, min.pct=0.25, logfc.threshold=0.25, test.use="MAST") ;
```



`test.use` Denotes which test to use. Available options are:

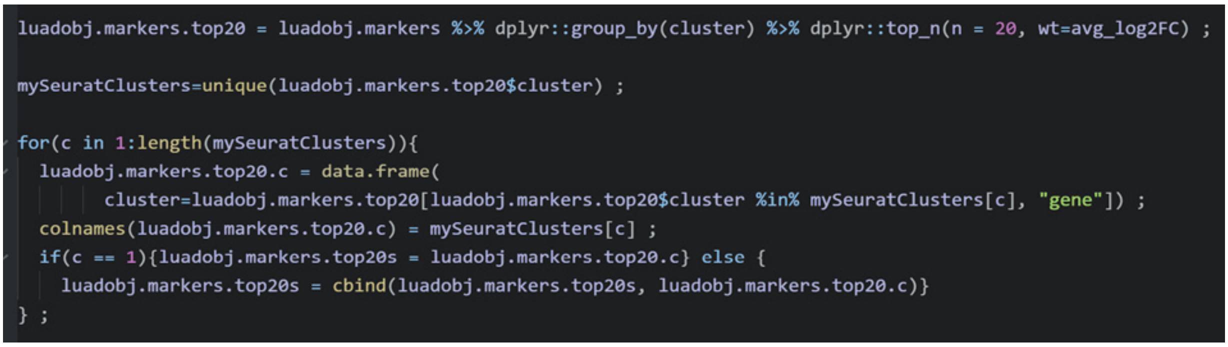
- "wilcox" : Identifies differentially expressed genes between two groups of cells using a Wilcoxon Rank Sum test (default)
- "bimod" : Likelihood-ratio test for single cell gene expression, (McDavid et al., Bioinformatics, 2013)
- "roc" : Identifies 'markers' of gene expression using ROC analysis. For each gene, evaluates (using AUC) a classifier built on that gene alone, to classify between two groups of cells. An AUC value of 1 means that expression values for this gene alone can perfectly classify the two groupings (i.e. Each of the cells in cells.1 exhibit a higher level than each of the cells in cells.2). An AUC value of 0 also means there is perfect classification, but in the other direction. A value of 0.5 implies that the gene has no predictive power to classify the two groups. Returns a 'predictive power' (abs(AUC-0.5) * 2) ranked matrix of putative differentially expressed genes.
- "t" : Identify differentially expressed genes between two groups of cells using the Student's t-test.
- "negbinom" : Identifies differentially expressed genes between two groups of cells using a negative binomial generalized linear model. Use only for UMI-based datasets
- "poisson" : Identifies differentially expressed genes between two groups of cells using a poisson generalized linear model. Use only for UMI-based datasets
- "LR" : Uses a logistic regression framework to determine differentially expressed genes. Constructs a logistic regression model predicting group membership based on each feature individually and compares this to a null model with a likelihood ratio test.
- "MAST" : Identifies differentially expressed genes between two groups of cells using a hurdle model tailored to scRNA-seq data. Utilizes the MAST package to run the DE testing.
- "DESeq2" : Identifies differentially expressed genes between two groups of cells based on a model using DESeq2 which uses a negative binomial distribution (Love et al, Genome Biology, 2014).This test does not support pre-filtering of genes based on average difference (or percent detection rate) between cell groups. However, genes may be pre-filtered based on their minimum detection rate (min.pct) across both cell groups. To use this method, please install DESeq2, using the instructions at https://bioconductor.org/packages/release/bioc/html/DESeq2.html

Identification of cluster-specific markers

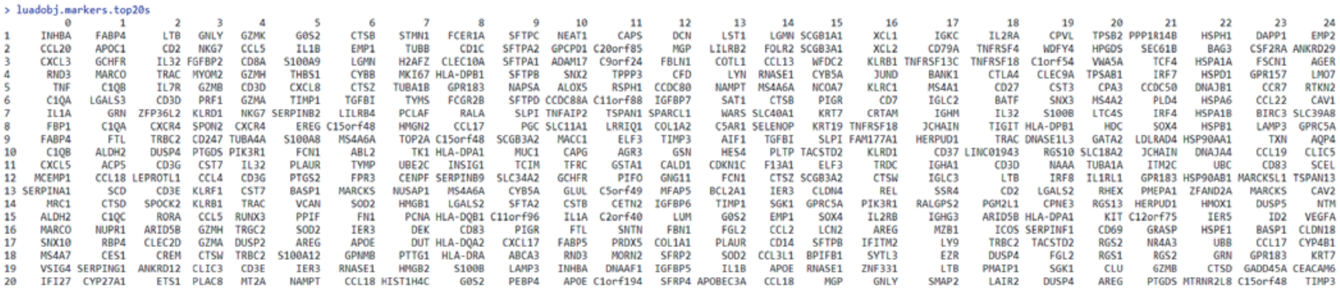


```
> head(luadobj.markers, 30)
           p_val avg_log2FC pct.1 pct.2 p_val_adj cluster       gene
INHBA          0   2.027373 0.957 0.324         0       0      INHBA
CCL20          0   1.948472 0.808 0.318         0       0      CCL20
CXCL3          0   1.835299 0.997 0.490         0       0      CXCL3
RND3           0   1.760417 0.711 0.210         0       0       RND3
TNF            0   1.717851 0.907 0.331         0       0        TNF
C1QA           0   1.593701 1.000 0.519         0       0       C1QA
IL1A           0   1.531175 0.692 0.180         0       0       IL1A
FBP1           0   1.530024 0.998 0.484         0       0       FBP1
FABP4          0   1.520869 0.967 0.406         0       0      FABP4
C1QB           0   1.489668 0.996 0.493         0       0       C1QB
CXCL5          0   1.463187 0.541 0.130         0       0      CXCL5
MCEMP1         0   1.349559 0.991 0.358         0       0     MCEMP1
SERPINA1       0   1.324133 0.998 0.501         0       0   SERPINA1
MRC1           0   1.294227 0.996 0.403         0       0       MRC1
ALDH2          0   1.290682 0.998 0.543         0       0      ALDH2
MARCO          0   1.281677 0.997 0.444         0       0      MARCO
SNX10          0   1.267388 0.989 0.432         0       0      SNX10
MS4A7          0   1.240686 0.998 0.449         0       0      MS4A7
VSIG4          0   1.233653 0.988 0.374         0       0      VSIG4
AC026369.3     0   1.210307 0.910 0.258         0       0 AC026369.3
LPL            0   1.186042 0.909 0.286         0       0        LPL
FTL            0   1.184815 1.000 0.996         0       0        FTL
C1QC           0   1.181961 0.989 0.374         0       0       C1QC
OLR1           0   1.164877 0.994 0.416         0       0       OLR1
STXBP2         0   1.144131 0.937 0.414         0       0     STXBP2
HLA-DRB5       0   1.140811 0.998 0.637         0       0   HLA-DRB5
LGALS3         0   1.119919 1.000 0.711         0       0     LGALS3
RETN           0   1.119641 0.794 0.301         0       0       RETN
MSR1           0   1.118809 0.983 0.391         0       0       MSR1
SERPING1       0   1.107077 0.944 0.352         0       0   SERPING1
```

Identification of cluster-specific markers

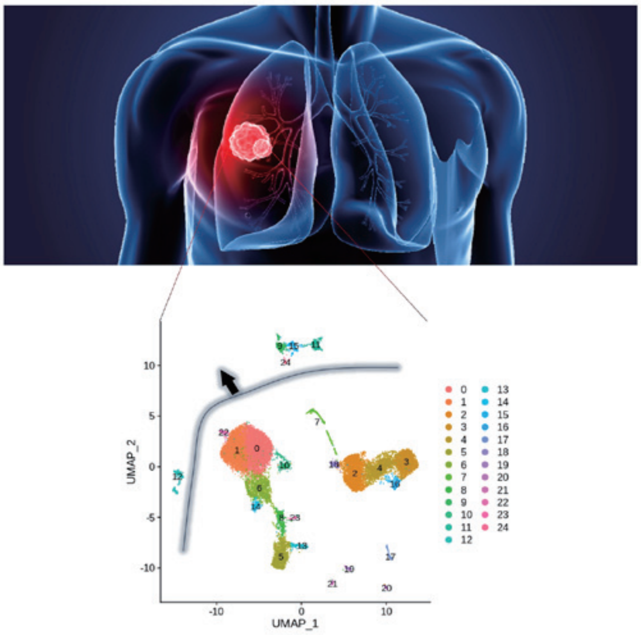


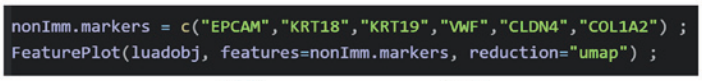
```
luadobj.markers.top20 = luadobj.markers %>% dplyr::group_by(cluster) %>% dplyr::top_n(n = 20, wt=avg_log2FC) ;

mySeuratClusters=unique(luadobj.markers.top20$cluster) ;

for(c in 1:length(mySeuratClusters)){
  luadobj.markers.top20.c = data.frame(
        cluster=luadobj.markers.top20[luadobj.markers.top20$cluster %in% mySeuratClusters[c], "gene"]) ;
  colnames(luadobj.markers.top20.c) = mySeuratClusters[c] ;
  if(c == 1){luadobj.markers.top20s = luadobj.markers.top20.c} else {
    luadobj.markers.top20s = cbind(luadobj.markers.top20s, luadobj.markers.top20.c)}
} ;
```



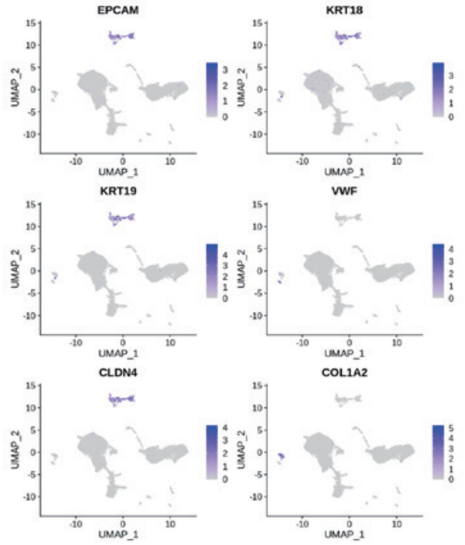
```
> luadobj.markers.top20s
          0        1        2      3      4        5        6        7        8        9      10       11      12       13      14      15       16        17        18       19      20       21       22       23      24
1     INHBA    FABP4      LTB   GNLY   GZMK     G0S2     CTSB    STMN1   FCER1A    SFTPC   NEAT1     CAPS     DCN     LST1    LGMN SCGB1A1     XCL1      IGKC     IL2RA     CPVL   TPSB2 PPP1R14B    HSPH1    DAPP1    EMP2
2     CCL20    APOC1      CD2   NKG7   CCL5     IL1B     EMP1     TUBB     CD1C   SFTPA2  GPCPD1 C20orf85     MGP   LILRB2   FOLR2 SCGB3A1     XCL2     CD79A   TNFRSF4    WDFY4   HPGDS   SEC61B     BAG3   CSF2RA ANKRD29
3     CXCL3    GCHFR     IL32 FGFBP2   CD8A   S100A9     LGMN    H2AFZ  CLEC10A   SFTPA1  ADAM17  C9orf24   FBLN1    COTL1   CCL13   WFDC2    KLRB1 TNFRSF13C  TNFRSF18  C1orf54   VWA5A     TCF4   HSPA1A    FSCN1    AGER
4      RND3    MARCO     TRAC  MYOM2   GZMH    THBS1     CYBB    MKI67 HLA-DPB1    SFTPB    SNX2    TPPP3     CFD      LYN  RNASE1   CYB5A     JUND     BANK1     CTLA4   CLEC9A  TPSAB1     IRF7    HSPD1   GPR157    LMO7
5       TNF     C1QB     IL7R   GZMB   CD3D    CXCL8     CTSZ   TUBA1B   GPR183    NAPSA   ALOX5    RSPH1  CCDC80    NAMPT  MS4A6A   NCOA7    KLRC1     MS4A1      CD27     CST3    CPA3   CCDC50   DNAJB1     CCR7   RTKN2
6      C1QA   LGALS3     CD3D   PRF1   GZMA    TIMP1    TGFBI     TYMS   FCGR2B    SFTPD CCDC88A C11orf88  IGFBP7     SAT1    CTSB    PIGR      CD7     IGLC2      BATF     SNX3   MS4A2     PLD4    HSPA6    CCL22    CAV1
7      IL1A      GRN  ZFP36L2  KLRD1   NKG7 SERPINB2   LILRB4    PCLAF     RALA     SLPI TNFAIP2   TSPAN1 SPARCL1     WARS SLC40A1    KRT7    CRTAM      IGHM      IL32    S100B   LTC4S     IRF4   HSPA1B    BIRC3 SLC39A8
8      FBP1     C1QA    CXCR4  SPON2  CXCR4     EREG C15orf48    HMGN2    CCL17      PGC SLC11A1   LRRIQ1  COL1A2    C5AR1 SELENOP   KRT19 TNFRSF18    JCHAIN     TIGIT HLA-DPB1     HDC     SOX4    HSPB1    LAMP3  GPRC5A
9     FABP4      FTL    TRBC2  CD247 TUBA4A   S100A8   MS4A6A    TOP2A C15orf48  SCGB3A2   MACC1     ELF3   TIMP3     AIF1   TGFBI    SLPI FAM177A1   HERPUD1      TRAC DNASE1L3   GATA2  LDLRAD4 HSP90AA1      TXN    AQP4
10     C1QB    ALDH2    DUSP4  PTGDS PIK3R1     FCN1     ABL2      TK1 HLA-DPA1     MUC1    CAPG     AGR3     GSN     HES4    PLTP TACSTD2    KLRD1      CD37 LINC01943    RGS10 SLC18A2   JCHAIN   DNAJA4    CCL19   CLIC5
11    CXCL5     ACP5     CD3G   CST7   IL32    PLAUR     TYMP    UBE2C   INSIG1     TCIM    TFRC    GSTA1   CALD1   CDKN1C   F13A1    ELF3     TRDC     IGHA1      CD3D     NAAA  TUBA1A    ITM2C      UBC     CD83    SCEL
12   MCEMP1    CCL18 LEPROTL1   CCL4   CD3G    PTGS2     FPR3    CENPF SERPINB9  SLC34A2   GCHFR     PIFO   GNG11     FCN1    CTSZ SCGB3A2     CTSW     IGLC3       LTB     IRF8  IL1RL1   GPR183 HSP90AB1 MARCKSL1 TSPAN13
13 SERPINA1      SCD     CD3E  KLRF1   CST7    BASP1   MARCKS   NUSAP1   MS4A6A    CYB5A    GLUL  C5orf49   MFAP5   BCL2A1    IER3   CLDN4      REL      SSR4       CD2   LGALS2    RHEX   PMEPA1  ZFAND2A   MARCKS    CAV2
14     MRC1     CTSD   SPOCK2  KLRB1   TRAC     VCAN     SOD2    HMGB1   LGALS2    SFTA2    CSTB    CETN2  IGFBP6    TIMP1    SGK1  GPRC5A   PIK3R1   RALGPS2    PGM2L1    CPNE3   RGS13  HERPUD1    HMOX1    DUSP5     NTM
15    ALDH2     C1QC     RORA   CCL5  RUNX3     PPIF      FN1     PCNA HLA-DQB1 C11orf96    IL1A  C2orf40     LUM     G0S2    EMP1    SOX4    IL2RB     IGHG3    ARID5B HLA-DPA1     KIT C12orf75     IER5      ID2   VEGFA
16    MARCO    NUPR1   ARID5B   GZMH  TRGC2     SOD2     IER3      DEK     CD83     PIGR     FTL     SNTN    FBN1     FGL2    CCL2    LCN2     AREG      MZB1      ICOS SERPINF1    CD69    GRASP    HSPE1    BASP1  CLDN18
17    SNX10     RBP4   CLEC2D   GZMA  DUSP2     AREG     APOE      DUT HLA-DQA2   CXCL17   FABP5    PRDX5  COL1A1    PLAUR    CD14   SFTPB   IFITM2       LY9     TRBC2  TACSTD2    RGS2    NR4A3      UBB    CCL17  CYP4B1
18    MS4A7     CES1     CREM   CTSW  TRBC2  S100A12    GPNMB    PTTG1  HLA-DRA    ABCA3    RND3    MORN2   SFRP2     SOD2  CCL3L1  BPIFB1    SYTL3       EZR     DUSP4     FGL2    RGS1     RGS2      GRN   GPR183    KRT7
19    VSIG4 SERPING1  ANKRD12  CLIC3   CD3E     IER3   RNASE1    HMGB2    S100B    LAMP3   INHBA   DNAAF1  IGFBP5     IL1B    APOE  RNASE1   ZNF331       LTB    PMAIP1     SGK1     CLU     GZMB     CTSD  GADD45A CEACAM6
20    IFI27  CYP27A1     ETS1  PLAC8   MT2A    NAMPT    CCL18 HIST1H4C     G0S2    PEBP4    APOE C1orf194   SFRP4 APOBEC3A   CCL18     MGP     GNLY     SMAP2     LAIR2    DUSP4    AREG    PTGDS MTRNR2L8 C15orf48   TIMP3
```

Discovery of cluster identity

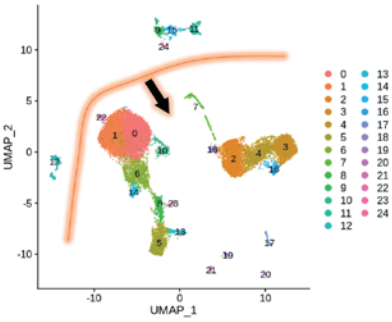


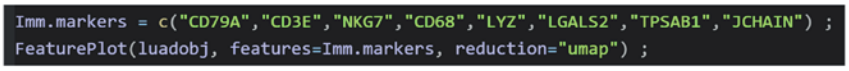


```
nonImm.markers = c("EPCAM","KRT18","KRT19","VWF","CLDN4","COL1A2") ;
FeaturePlot(luadobj, features=nonImm.markers, reduction="umap") ;
```

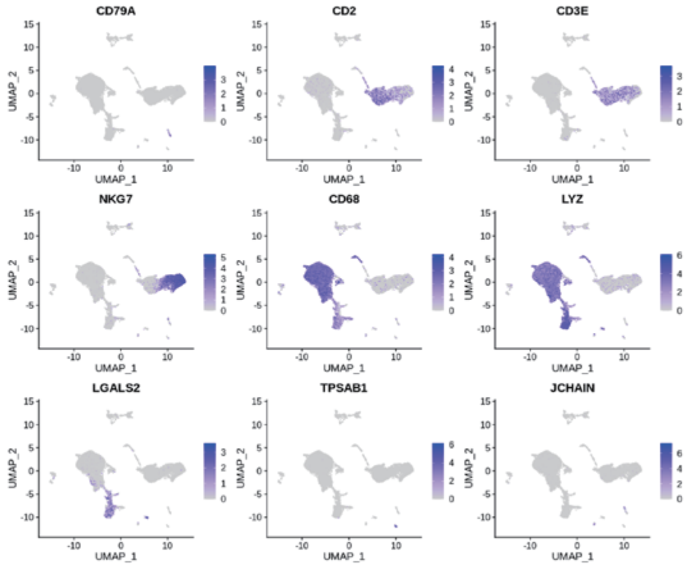


Discovery of cluster identity

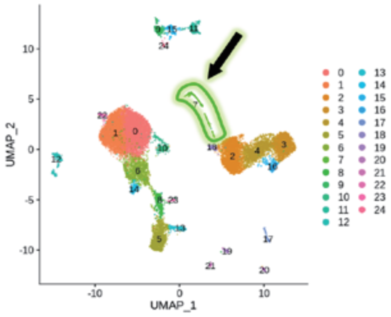


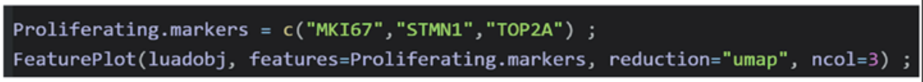


```
Imm.markers = c("CD79A","CD3E","NKG7","CD68","LYZ","LGALS2","TPSAB1","JCHAIN") ;
FeaturePlot(luadobj, features=Imm.markers, reduction="umap") ;
```

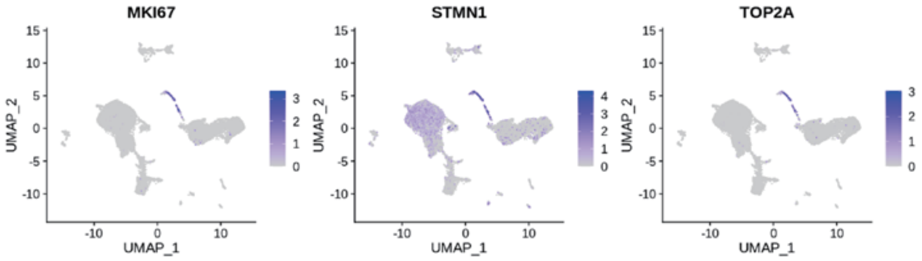


Discovery of cluster identity



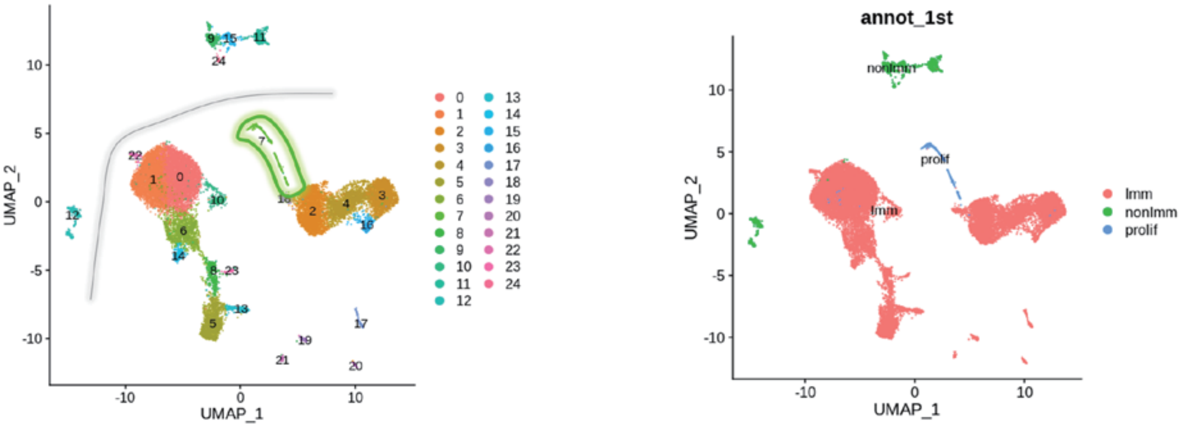


```
Proliferating.markers = c("MKI67","STMN1","TOP2A") ;
FeaturePlot(luadobj, features=Proliferating.markers, reduction="umap", ncol=3) ;
```

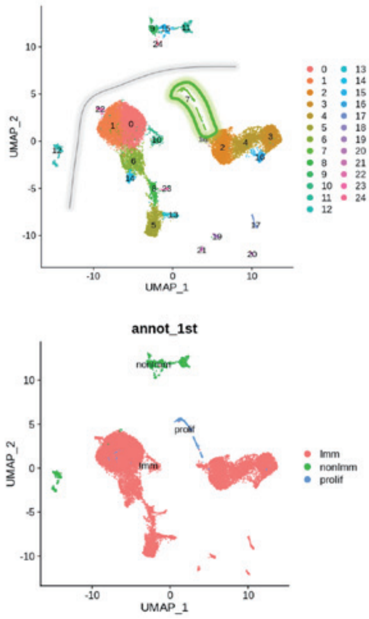


Discovery of cluster identity

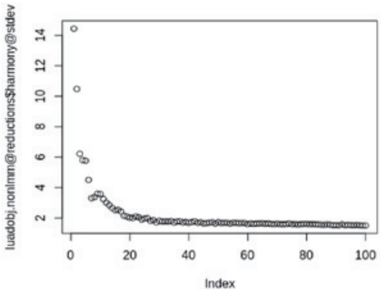
```
luadobj$annot_1st = "" ;
luadobj@meta.data[luadobj@meta.data$seurat_clusters %in% c(9,11,15,24, 12), ]$annot_1st <- "nonImm" ;
luadobj@meta.data[luadobj@meta.data$seurat_clusters %in% c(0:6,8,10,13,14,16:23), ]$annot_1st <- "Imm" ;
luadobj@meta.data[luadobj@meta.data$seurat_clusters %in% c(7), ]$annot_1st <- "prolif" ;

Idents(luadobj) = "annot_1st" ;
DimPlot(luadobj, group.by = "annot_1st", reduction="umap", label=T) ;
```



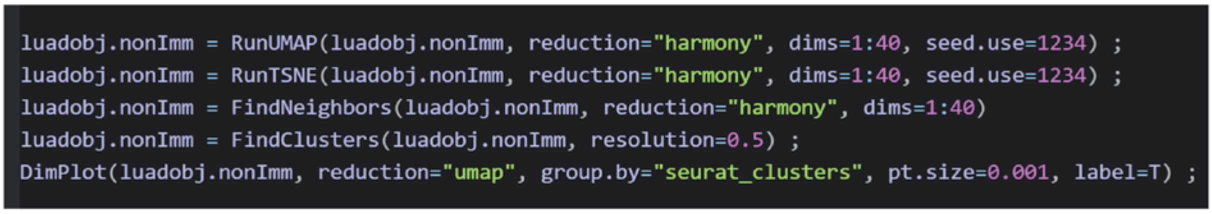
Discovery of sub-cluster identity



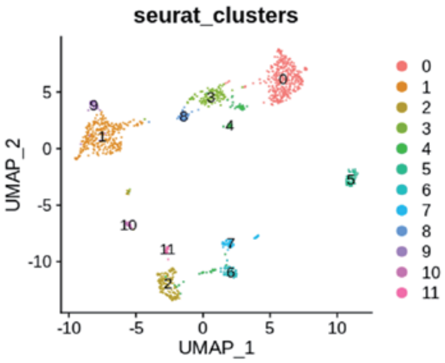
```
luadobj.nonImm = subset(luadobj, subset = seurat_clusters %in% c(9,11,15,24, 12)) ;

luadobj.nonImm = NormalizeData(luadobj.nonImm, normalization.method = "LogNormalize", scale.factor = 10000) ;
luadobj.nonImm = FindVariableFeatures(luadobj.nonImm, selection.method="vst", nfeatures=2000) ;
luadobj.nonImm = ScaleData(luadobj.nonImm, features=rownames(luadobj.nonImm)) ;
luadobj.nonImm = RunPCA(luadobj.nonImm, features=VariableFeatures(object=luadobj.nonImm), npcs=100) ;
luadobj.nonImm = RunHarmony(luadobj.nonImm, "orig.ident") ;
plot(luadobj.nonImm@reductions$harmony@stdev) ;
```



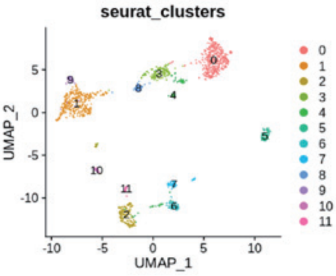
Discovery of sub-cluster identity

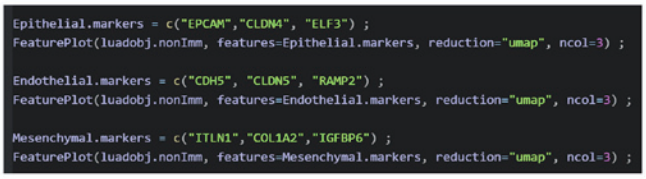


```
luadobj.nonImm = RunUMAP(luadobj.nonImm, reduction="harmony", dims=1:40, seed.use=1234) ;
luadobj.nonImm = RunTSNE(luadobj.nonImm, reduction="harmony", dims=1:40, seed.use=1234) ;
luadobj.nonImm = FindNeighbors(luadobj.nonImm, reduction="harmony", dims=1:40)
luadobj.nonImm = FindClusters(luadobj.nonImm, resolution=0.5) ;
DimPlot(luadobj.nonImm, reduction="umap", group.by="seurat_clusters", pt.size=0.001, label=T) ;
```

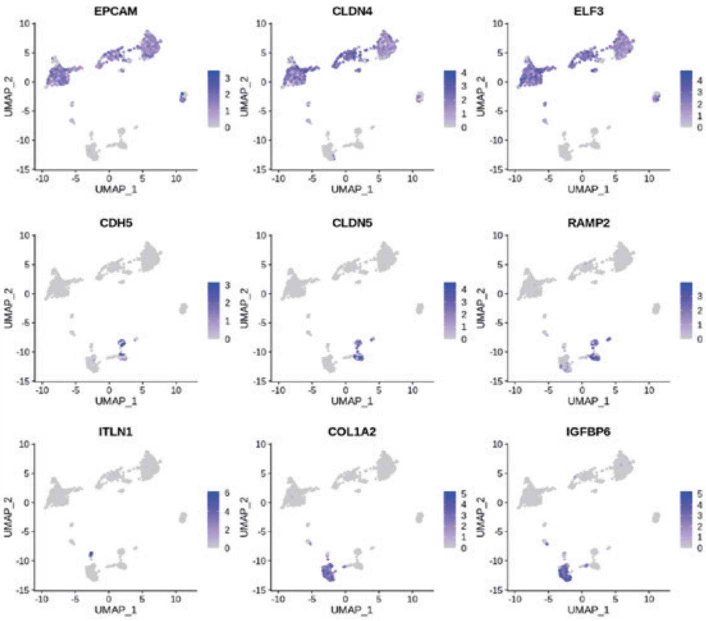


Discovery of sub-cluster identity

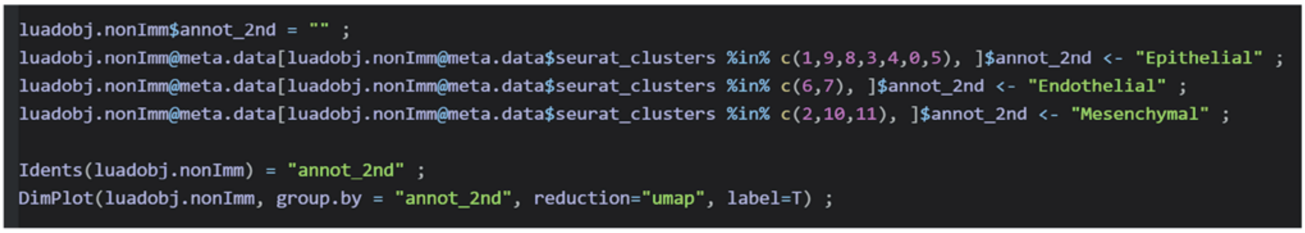




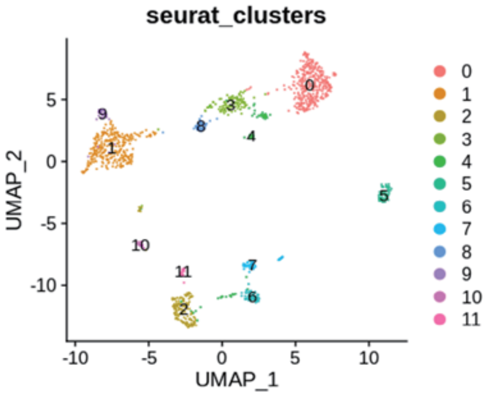
```
Epithelial.markers = c("EPCAM","CLDN4", "ELF3") ;
FeaturePlot(luadobj.nonImm, features=Epithelial.markers, reduction="umap", ncol=3) ;

Endothelial.markers = c("CDH5", "CLDN5", "RAMP2") ;
FeaturePlot(luadobj.nonImm, features=Endothelial.markers, reduction="umap", ncol=3) ;

Mesenchymal.markers = c("ITLN1","COL1A2","IGFBP6") ;
FeaturePlot(luadobj.nonImm, features=Mesenchymal.markers, reduction="umap", ncol=3) ;
```

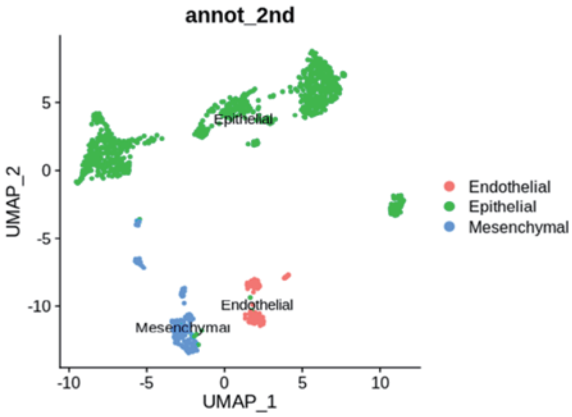


Discovery of sub-cluster identity

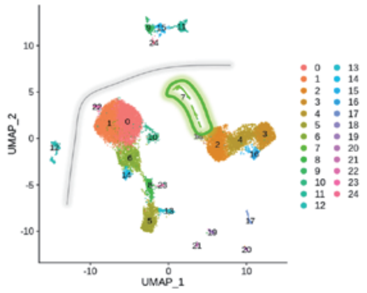


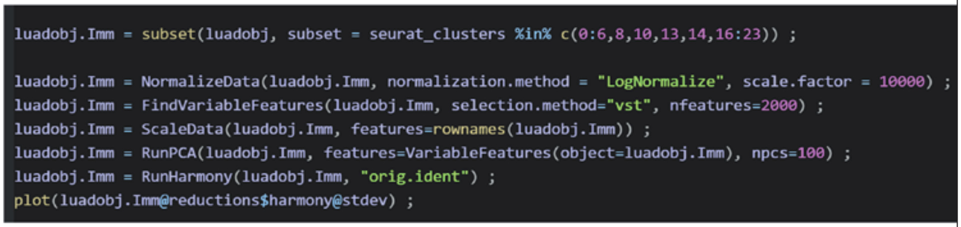
```
luadobj.nonImm$annot_2nd = "" ;
luadobj.nonImm@meta.data[luadobj.nonImm@meta.data$seurat_clusters %in% c(1,9,8,3,4,0,5), ]$annot_2nd <- "Epithelial" ;
luadobj.nonImm@meta.data[luadobj.nonImm@meta.data$seurat_clusters %in% c(6,7), ]$annot_2nd <- "Endothelial" ;
luadobj.nonImm@meta.data[luadobj.nonImm@meta.data$seurat_clusters %in% c(2,10,11), ]$annot_2nd <- "Mesenchymal" ;

Idents(luadobj.nonImm) = "annot_2nd" ;
DimPlot(luadobj.nonImm, group.by = "annot_2nd", reduction="umap", label=T) ;
```



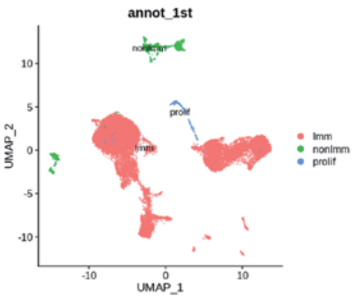


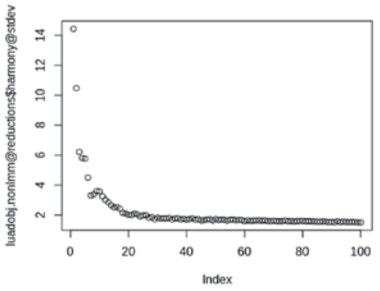
Discovery of sub-cluster identity



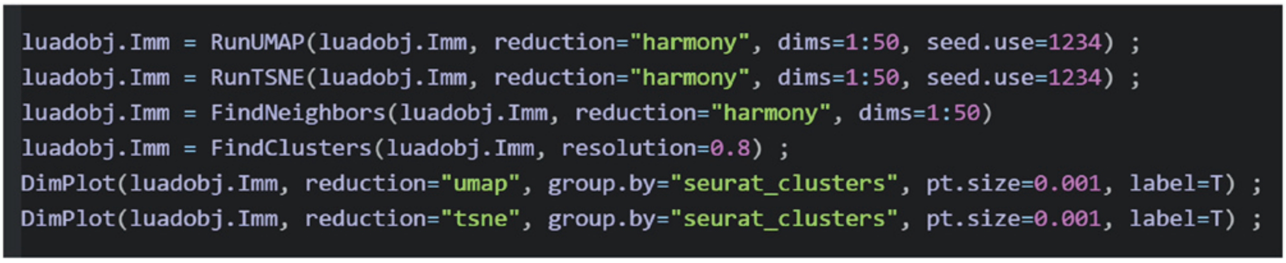


```
luadobj.Imm = subset(luadobj, subset = seurat_clusters %in% c(0:6,8,10,13,14,16:23)) ;

luadobj.Imm = NormalizeData(luadobj.Imm, normalization.method = "LogNormalize", scale.factor = 10000) ;
luadobj.Imm = FindVariableFeatures(luadobj.Imm, selection.method="vst", nfeatures=2000) ;
luadobj.Imm = ScaleData(luadobj.Imm, features=rownames(luadobj.Imm)) ;
luadobj.Imm = RunPCA(luadobj.Imm, features=VariableFeatures(object=luadobj.Imm), npcs=100) ;
luadobj.Imm = RunHarmony(luadobj.Imm, "orig.ident") ;
plot(luadobj.Imm@reductions$harmony@stdev) ;
```

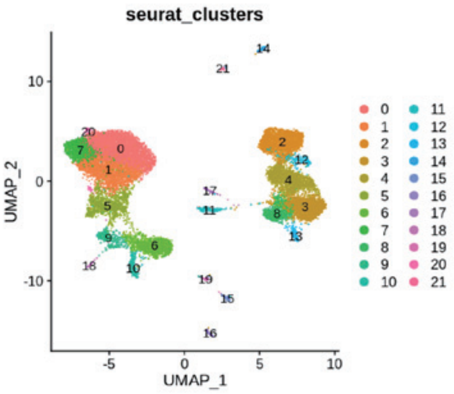


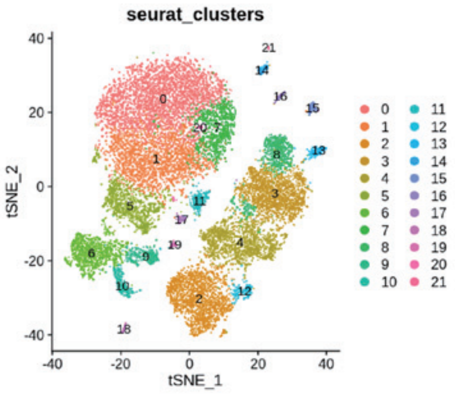


Discovery of sub-cluster identity

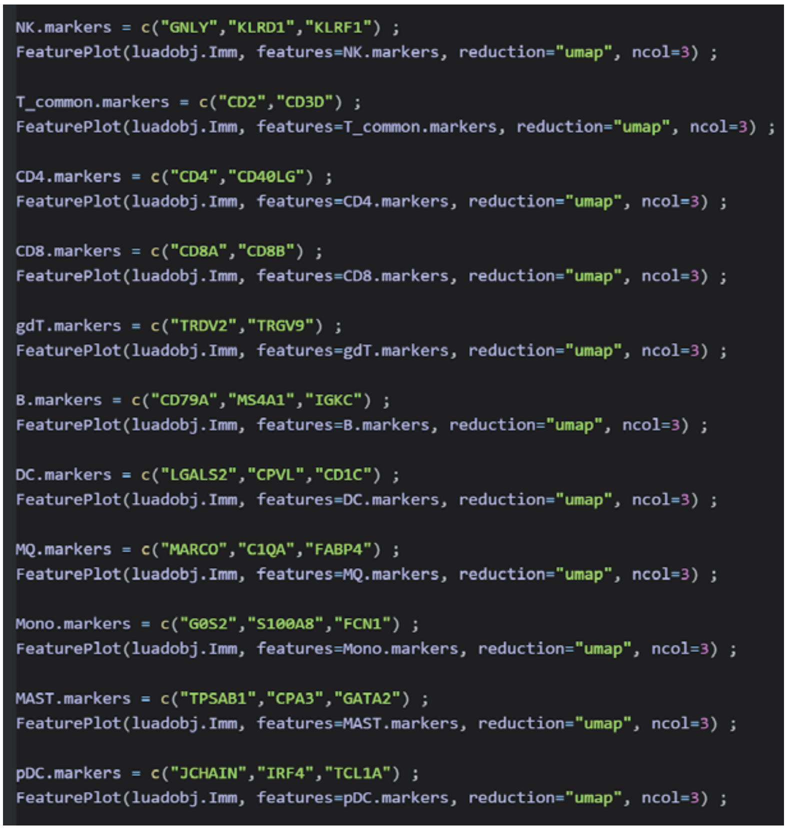


```
luadobj.Imm = RunUMAP(luadobj.Imm, reduction="harmony", dims=1:50, seed.use=1234) ;
luadobj.Imm = RunTSNE(luadobj.Imm, reduction="harmony", dims=1:50, seed.use=1234) ;
luadobj.Imm = FindNeighbors(luadobj.Imm, reduction="harmony", dims=1:50)
luadobj.Imm = FindClusters(luadobj.Imm, resolution=0.8) ;
DimPlot(luadobj.Imm, reduction="umap", group.by="seurat_clusters", pt.size=0.001, label=T) ;
DimPlot(luadobj.Imm, reduction="tsne", group.by="seurat_clusters", pt.size=0.001, label=T) ;
```



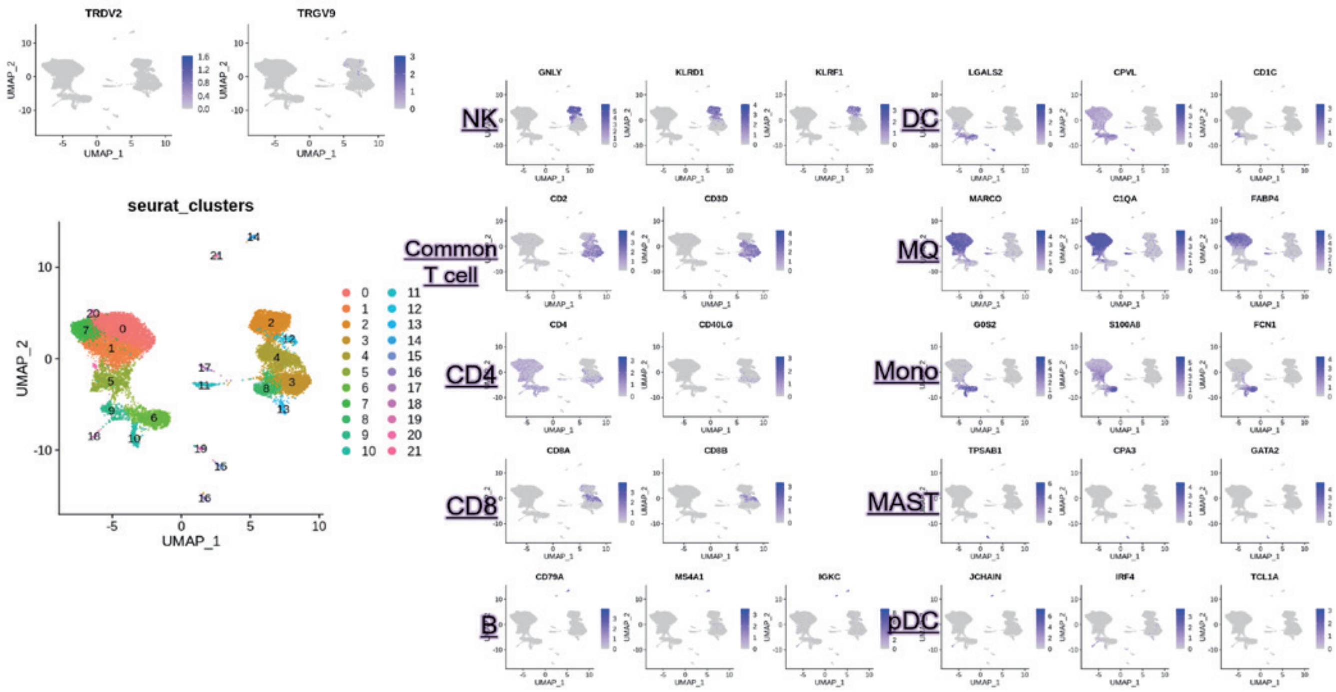


Discovery of sub-cluster identity

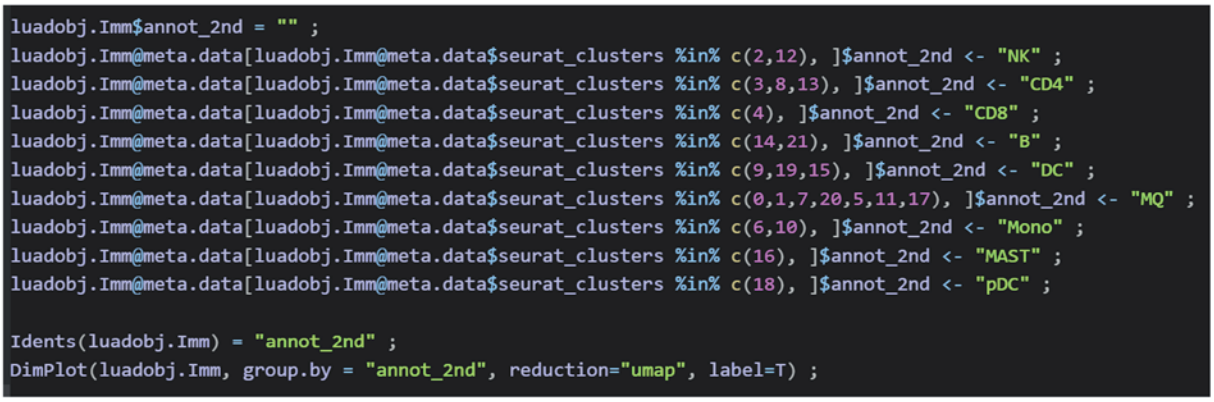


```
NK.markers = c("GNLY","KLRD1","KLRF1") ;
FeaturePlot(luadobj.Imm, features=NK.markers, reduction="umap", ncol=3) ;

T_common.markers = c("CD2","CD3D") ;
FeaturePlot(luadobj.Imm, features=T_common.markers, reduction="umap", ncol=3) ;

CD4.markers = c("CD4","CD40LG") ;
FeaturePlot(luadobj.Imm, features=CD4.markers, reduction="umap", ncol=3) ;

CD8.markers = c("CD8A","CD8B") ;
FeaturePlot(luadobj.Imm, features=CD8.markers, reduction="umap", ncol=3) ;

gdT.markers = c("TRDV2","TRGV9") ;
FeaturePlot(luadobj.Imm, features=gdT.markers, reduction="umap", ncol=3) ;

B.markers = c("CD79A","MS4A1","IGKC") ;
FeaturePlot(luadobj.Imm, features=B.markers, reduction="umap", ncol=3) ;

DC.markers = c("LGALS2","CPVL","CD1C") ;
FeaturePlot(luadobj.Imm, features=DC.markers, reduction="umap", ncol=3) ;

MQ.markers = c("MARCO","C1QA","FABP4") ;
FeaturePlot(luadobj.Imm, features=MQ.markers, reduction="umap", ncol=3) ;

Mono.markers = c("G0S2","S100A8","FCN1") ;
FeaturePlot(luadobj.Imm, features=Mono.markers, reduction="umap", ncol=3) ;

MAST.markers = c("TPSAB1","CPA3","GATA2") ;
FeaturePlot(luadobj.Imm, features=MAST.markers, reduction="umap", ncol=3) ;

pDC.markers = c("JCHAIN","IRF4","TCL1A") ;
FeaturePlot(luadobj.Imm, features=pDC.markers, reduction="umap", ncol=3) ;
```

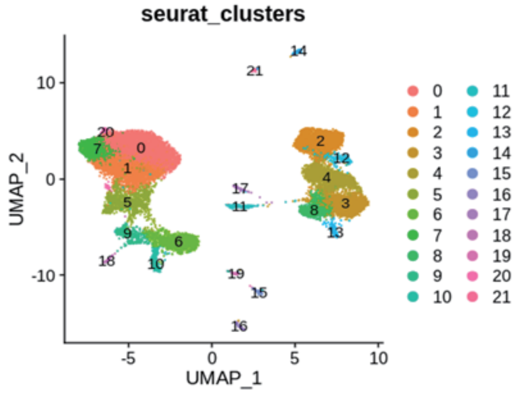
Discovery of sub-cluster identity

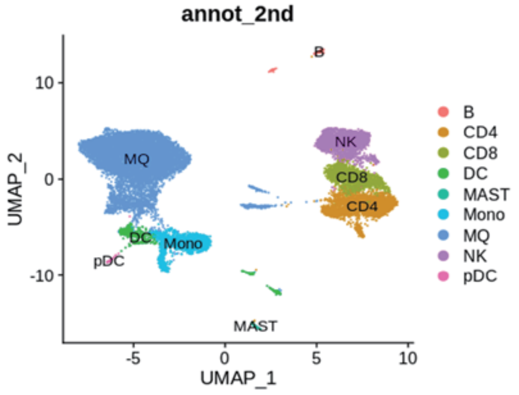


Discovery of sub-cluster identity

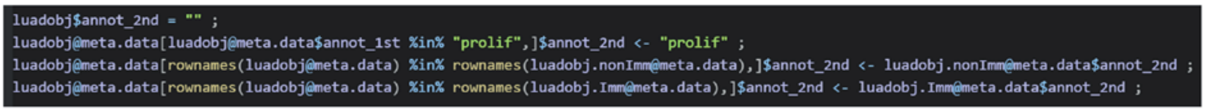


```
luadobj.Imm$annot_2nd = "" ;
luadobj.Imm@meta.data[luadobj.Imm@meta.data$seurat_clusters %in% c(2,12), ]$annot_2nd <- "NK" ;
luadobj.Imm@meta.data[luadobj.Imm@meta.data$seurat_clusters %in% c(3,8,13), ]$annot_2nd <- "CD4" ;
luadobj.Imm@meta.data[luadobj.Imm@meta.data$seurat_clusters %in% c(4), ]$annot_2nd <- "CD8" ;
luadobj.Imm@meta.data[luadobj.Imm@meta.data$seurat_clusters %in% c(14,21), ]$annot_2nd <- "B" ;
luadobj.Imm@meta.data[luadobj.Imm@meta.data$seurat_clusters %in% c(9,19,15), ]$annot_2nd <- "DC" ;
luadobj.Imm@meta.data[luadobj.Imm@meta.data$seurat_clusters %in% c(0,1,7,20,5,11,17), ]$annot_2nd <- "MQ" ;
luadobj.Imm@meta.data[luadobj.Imm@meta.data$seurat_clusters %in% c(6,10), ]$annot_2nd <- "Mono" ;
luadobj.Imm@meta.data[luadobj.Imm@meta.data$seurat_clusters %in% c(16), ]$annot_2nd <- "MAST" ;
luadobj.Imm@meta.data[luadobj.Imm@meta.data$seurat_clusters %in% c(18), ]$annot_2nd <- "pDC" ;

Idents(luadobj.Imm) = "annot_2nd" ;
DimPlot(luadobj.Imm, group.by = "annot_2nd", reduction="umap", label=T) ;
```

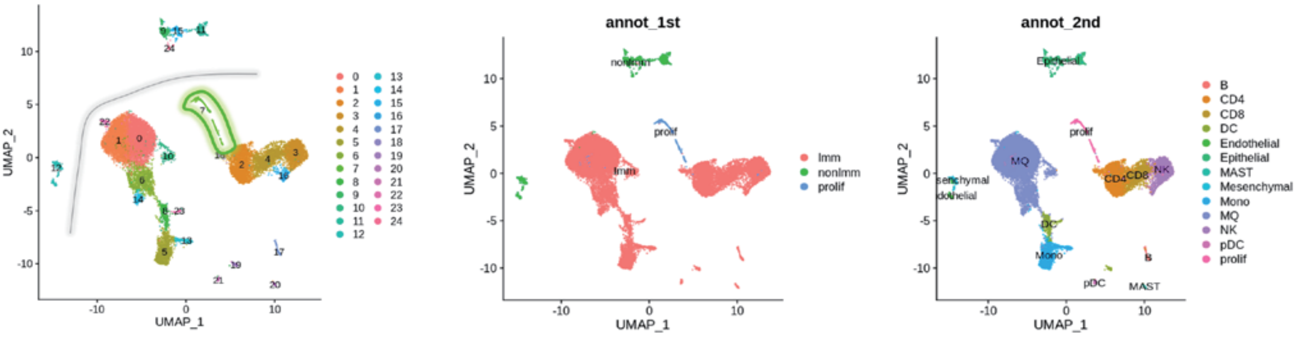




Discovery of sub-cluster identity



```
luadobj$annot_2nd = "" ;
luadobj@meta.data[luadobj@meta.data$annot_1st %in% "prolif",]$annot_2nd <- "prolif" ;
luadobj@meta.data[rownames(luadobj@meta.data) %in% rownames(luadobj.nonImm@meta.data),]$annot_2nd <- luadobj.nonImm@meta.data$annot_2nd ;
luadobj@meta.data[rownames(luadobj@meta.data) %in% rownames(luadobj.Imm@meta.data),]$annot_2nd <- luadobj.Imm@meta.data$annot_2nd ;
```



Thank you!

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