# SCODA pipeline

for single-cell transcriptomics data analysis

**MLBI** Lab

### SCODA?

- Web-based, Fully-automated, all-in-one computing service for Single-Cell (transcript)Omics Data Analysis (single-cell RNA-seq)
- ☐ It is useful for
  - Tissue/tumor microenvironment study to elucidate underlying biological mechanism
  - O Discovery of therapeutic targets in many diseases & cancers

### **How SCODA works?**

Web-based, automated analysis pipeline: SCODA MLBI SCODA pipeline **Functional Analysis Cell-type annotation** Discover the Hidden Secrets in Your Single-Cell Tr Our Cutting-Edge Analysis pip **CNV** estimation Identifying tumor cells **Cell-cell interaction** Diff. expressed gene **Gene set analysis** Marker discovery . Download an exampte dataset hom.

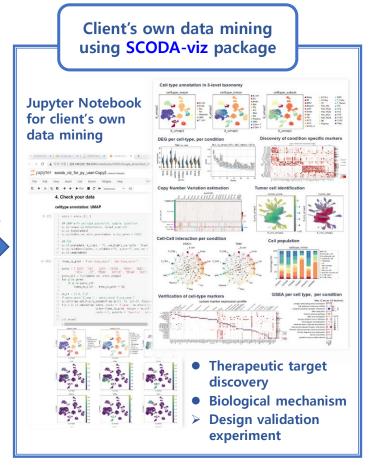
Lupload it through the form below.

Cince it is done, download the result (xxx.tar.gz).

Open Jupyter notebook in Google Colab by clicking h

Upload the xxx.tar.gz file and follow the instruction th Mandatory input N. GENES, MN = 200 N. CILLS, MN = 10 PCT CORNT MI, MAX = 20 REFERENCE, CELLTHYSE, FOR, TUMOR, II = IT cell, Tell, Thylide, Flavebland, IN LAM, CELLS, FOR, CCI = 40 CO, MN, OC, MPG, TO, COLWIT = 05 CO, MN, ACT, MPG, TO, COLWIT = 05 CO, PEG, BASE = cellspape, mice' MN, JAM, CELLS, FOR, DCG = 100 DGS, MPS, GROUP = 01 DGS, MPS, GROUP = 01 DGS, MPS, GROUP = 01 **Cell-type annotation** CNV & ploidy inference (InferCNVpy + InferPloidy) Cell-cell interaction (SCANpy + GSEApy) Visualization & mlbi-lab.net mlbi-lab.com

Download result file with functional annotations



Single-cell

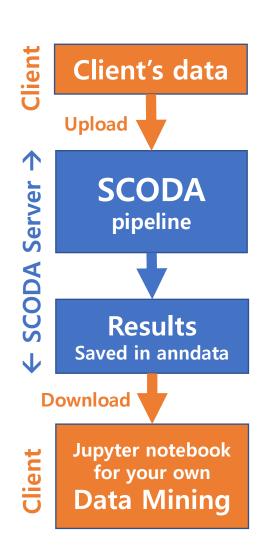
**RNA-seq** 

raw data

(Count matrix)

Upload raw data

### **How SCODA works?**



#### (1) Simply upload your data

- 1. Compressed 10x mtx files + optional meta data.csv
- 2. Compressed CSV file + optional meta\_data.csv
- 3. Compressed h5ad file (including meta data in obs field)

#### (2) Automated pipeline analyzes your data

- 1. Celltype annotation (HiCAT, MIT license)
- 2. DEG & GSA/GSEA (GSEApy, MIT license)
- 3. Cell-cell interaction (CellPhoneDB, MIT license)
- 4. Tumor cell identification (InferCNV, BSD-3c + InferPloidy, MIT)
- **❖** We use proven & standardized analysis tools
- \* The pipeline can be tailored with optional settings

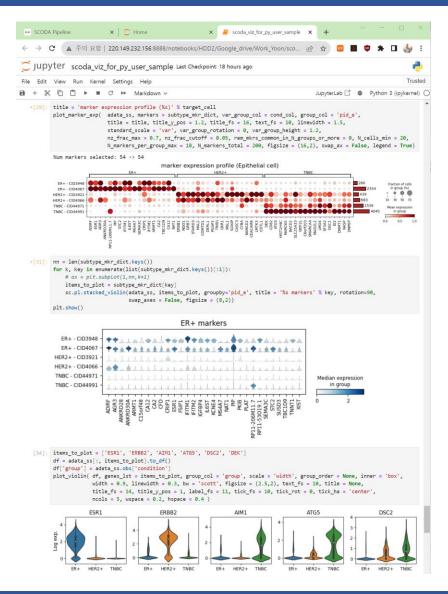
#### (3) Once it is done, you can download the result

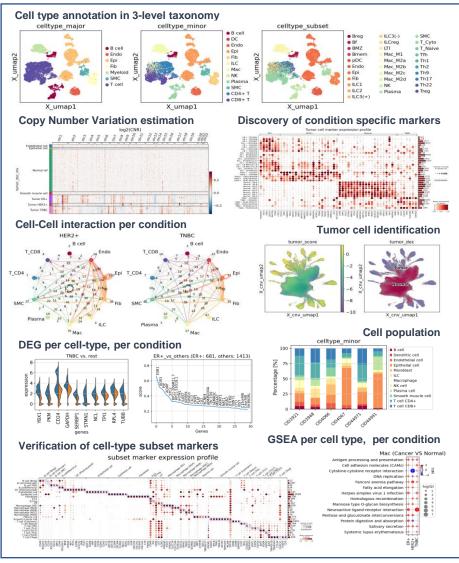
- 1. Saved in Anndata formatted h5ad file (tar.gz compressed)
- 2. It contains all the analysis results and the count matrix itself

#### (4) Explore the results by yourself on your web browser

- 1. We provide "jupyter notebook" with example codes
- 2. You can freely use SCODA-viz & SCANpy (free open SW)
- 3. With a little bit of programming, you can get deeper insight into the single-cell RNA-seq data you uploaded
- 4. We offer free workshop for data mining (upon request)

### Visualization & data mining





# **Using SCODA**

- □ SCODA homepage: <a href="https://mlbi-lab.net">https://mlbi-lab.net</a>
- MLBI lab company homepage: <a href="https://mlbi-lab.com">https://mlbi-lab.com</a>

# Why SCODA?

**Bio/Medical background** 



**SCODA** 

with SCODA-viz tool

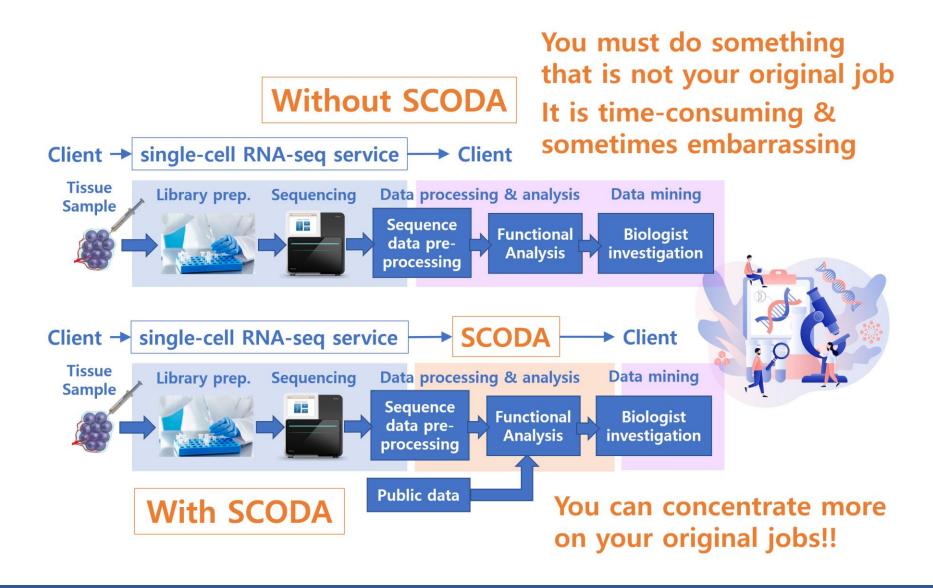


Little bit of programming skill (Free Training workshop available)



Single-cell RNA-seq Data analysis expert

# Why SCODA?



### Related papers

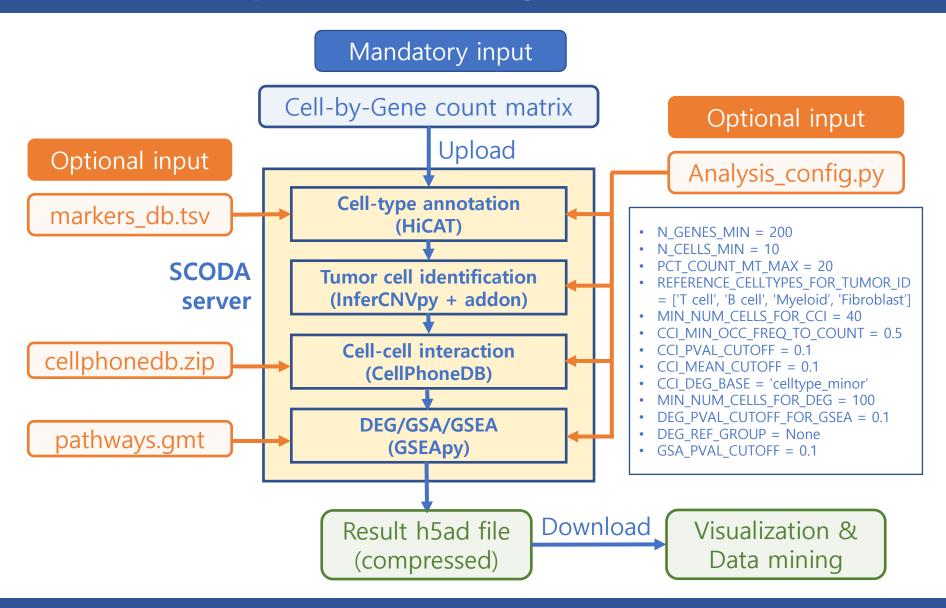
#### □ HiCAT

O Hierarchical cell-type identifier accurately distinguishes immune-cell subtypes enabling precise profiling of tissue microenvironment with single-cell RNA-sequencing, **Briefings in bioinformatics**, **March 2023** 

### ☐ Studies using SCODA

- O Colon-Targeted eNAMPT-Specific Peptide Systems for Treatment of DSS-Induced Acute and Chronic Colitis in Mouse, **Antioxidants, Nov. 2022**
- O Integrative analysis of ulcerative colitis progression using single-cell RNA-seq and microbiome, **Communications Biology, June 2024**
- A Retrospective View on Triple Negative Breast Cancer Microenvironment: Novel Markers, Interactions, and Mechanisms of Tumor-Associated Components using public Single-cell RNA Seq Datasets, **Cancers, May 2024**

# SCODA Optional configuration



### **SCODA?**

#### □ SCODA is useful for

- In-vivo tissue/tumor micro-environment (TME) study.
- Immune cell profiling in many diseases, e.g., autoimmune disease & cancer
- O Discovery of diagnostic/prognostic markers
- Discovery of druggable targets and its biological mechanism around pathological tissue
- Exploring drug response and mechanism of action

### **☐** But not suitable yet for

- Studies with cell line
- Differentiation study

### **Summary**

- **□** SCODA utilizes proven open-source software
  - O HiCAT (MIT license) for cell type annotation
  - O CellPhoneDB (MIT license) for inferring cell-cell interaction
  - InferCNVpy (BSC-3clause) for CNV estimation
  - GSEApy (BSC-3clause) for gene set enrichment analysis
- □ SCODA-viz package and example jupyter notebook freely available for visualization and data mining
  - With a little bit of programming skill, you can create any kind of plots you want. (Free training workshop available upon request)
- ☐ It accelerate your research with single-cell RNA-seq experiment, saving your time and the cost.
  - Use SCODA first to get insight into the tissue of your interest.
  - Then, plan biological experiment to verify your hypothesis.

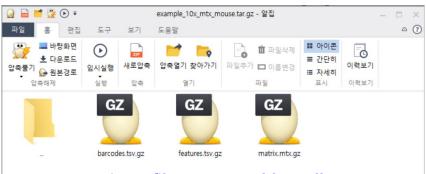
# Thank you

# Input data formatting (1) 10x\_mtx

### 1. Contents of the compressed input file (.zip or .gz)

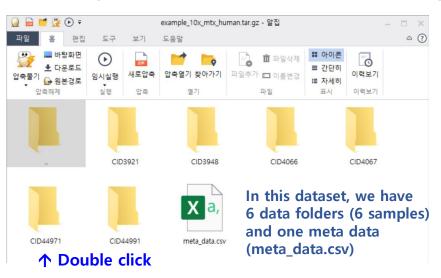


#### 3. Further opening each data folder, we see

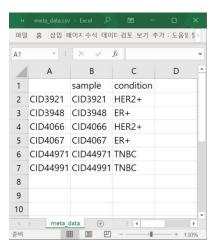


It contains 3 files generated by CellRanger

#### 2. Opening the main folder, we see the following



#### 4. Opening the meta\_data.csv



- A: Index column
  B: sample name
  C: condition
- Each index corresponds to one of the folders (name).
- But we don't require sample name must correspond to a folder name.
- Condition is required to perform DEG, GSEA, cell-cell interaction to compare difference among conditions

# Input data formatting (2) csv format

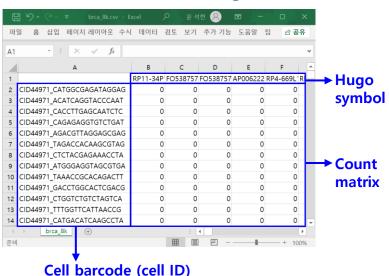
1. Contents of the compressed input file (.zip or .gz)



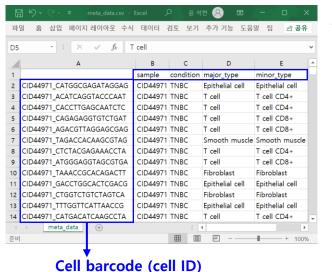
2. Opening the main folder, we see the following



3. Data csv file (containing count matrix)



4. meta\_data.csv 파일



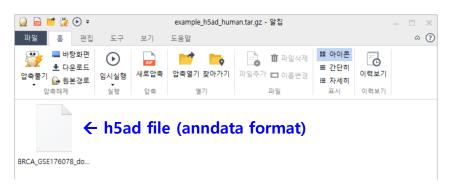
A: Index column
B: sample
C: condition

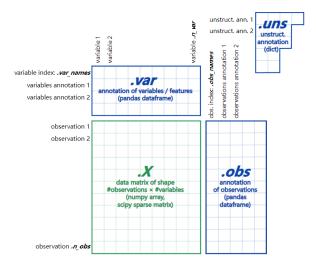
D~: optional items

- The indices of the data matrix must have 1-to-1 correspondence to those of meta data matrix.
- Condition is required to perform DEG, GSEA, cell-cell interaction to compare difference among conditions

# Input data formatting (3) h5ad format

#### 1. Contents of the compressed input file (.zip or .gz)





#### https://anndata.readthedocs.io/en/latest/

#### 2. h5ad file contents

```
adata_t = sc.read_h5ad(file_h5ad)
adata_t

AnnData object with n_obs × n_vars = 12000 × 29733
    obs: 'Patient', 'Percent_mito', 'nCount_RNA', 'nFeature_RNA', 'Celltype_Major', 'Celltype_Minor', 'Celltype_Subset', 'subtype', 'gene_module', 'Calls', 'normal_cell_call', 'CNA_value', 'sample', 'condition' var: 'gene_ids'
```

- AnnData contains "sample" and "condition" columns to run DEG/GSEA. DEG/GSEA will not be performed if the obs field does not contain both "sample" and "condition" column.
- If the "sample" column exists in the obs field, cell-cell interaction will be performed per-sample the same as in the above.