

A comprehensive single cell RNA analysis pipeline

Mingbo Cheng

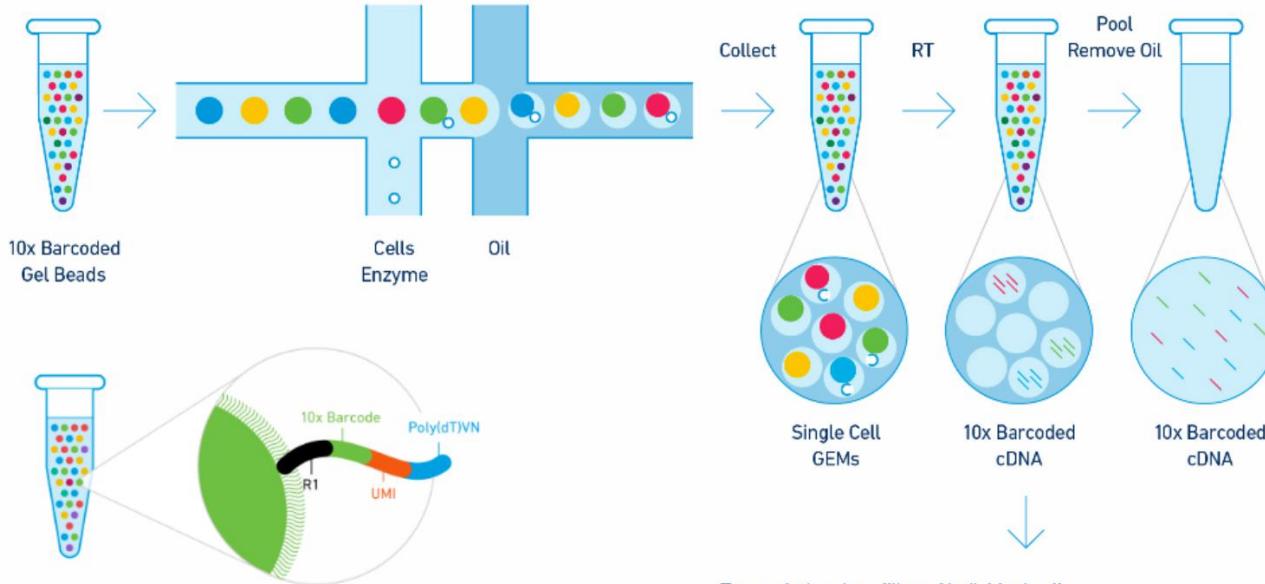
Outline

- Overview of data analysis in scRNAseq data
- Single cell pipeline introduction
 - overview
 - output example
 - quick start
 - parameter settings
 - frequently asked questions

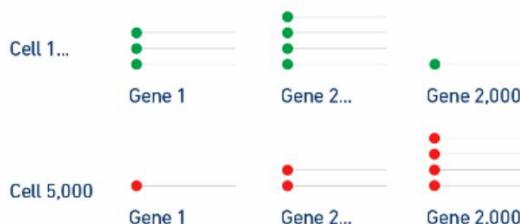
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10X single cell RNA analysis

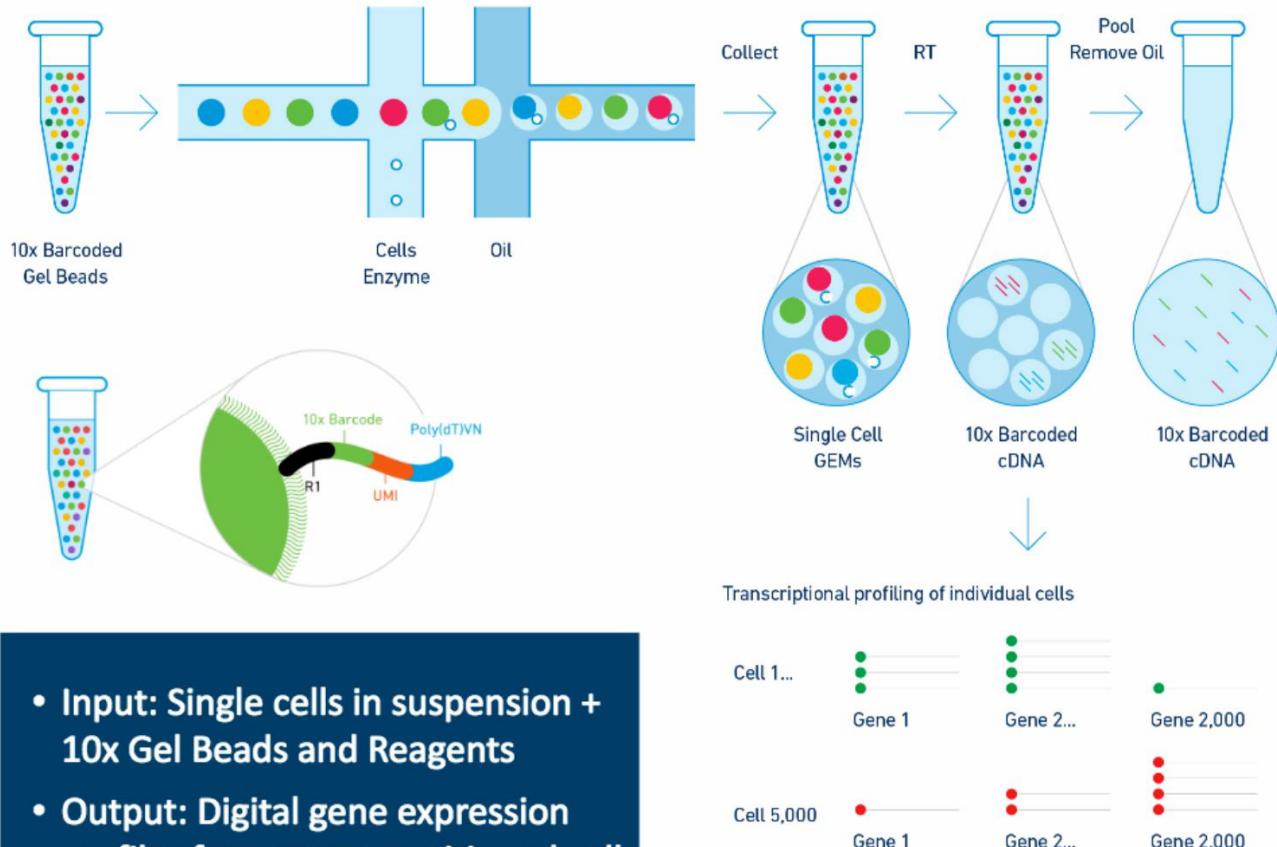


- Input: Single cells in suspension + 10x Gel Beads and Reagents
- Output: Digital gene expression profiles from every partitioned cell

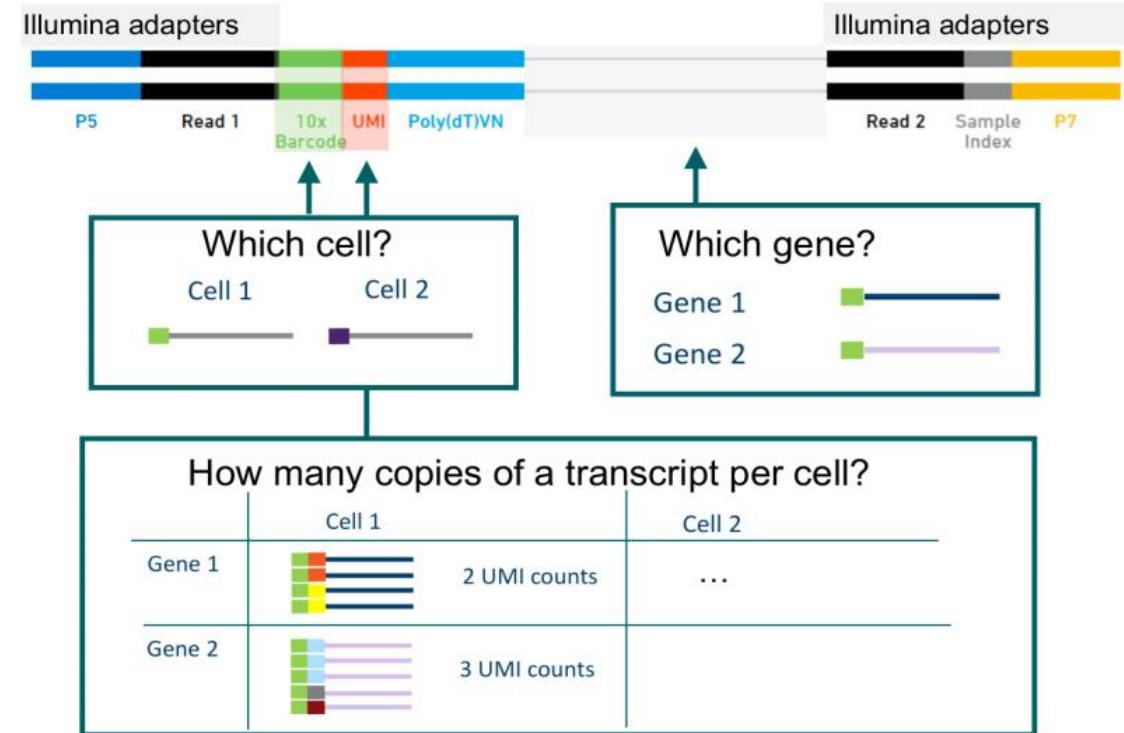


Source: 10x genomics

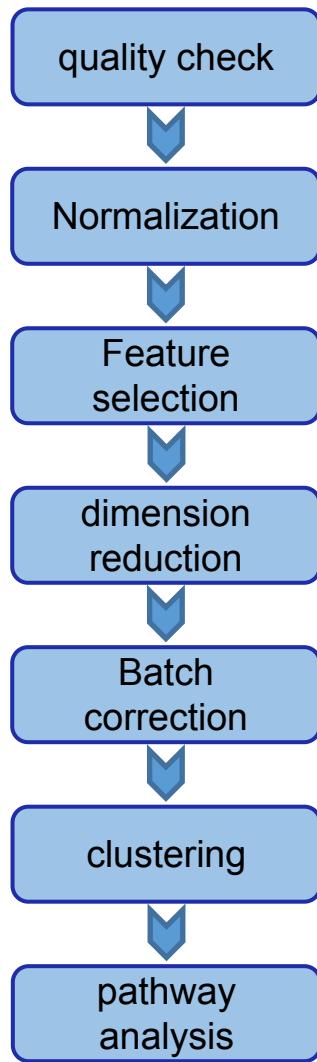
10X single cell RNA analysis



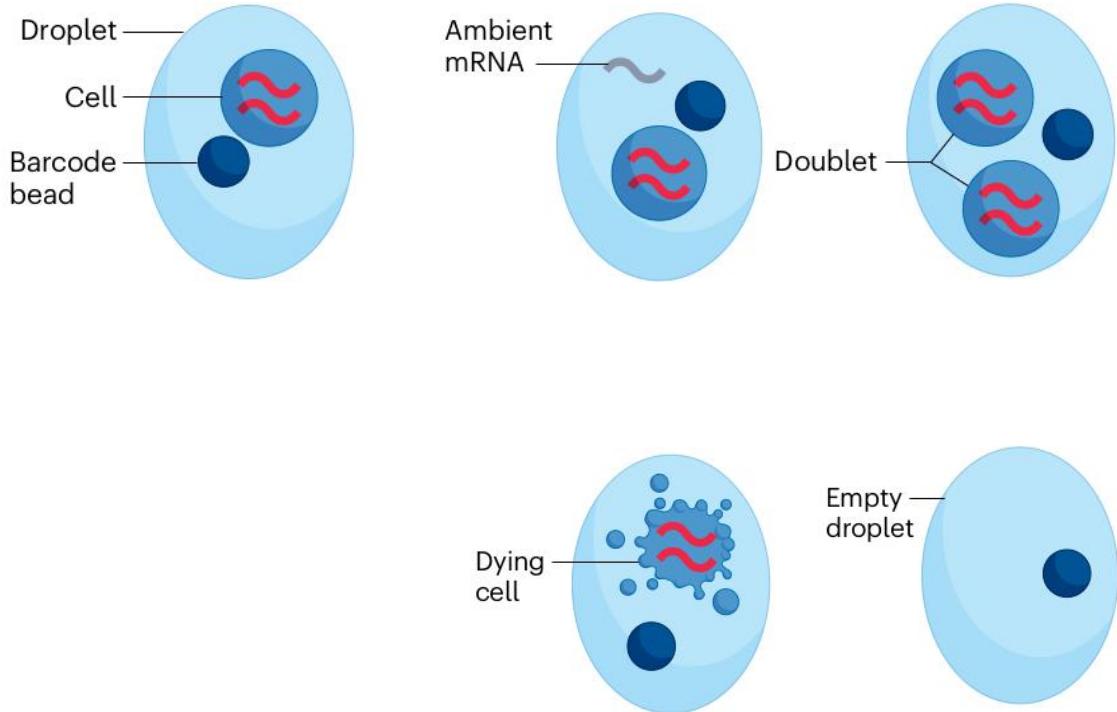
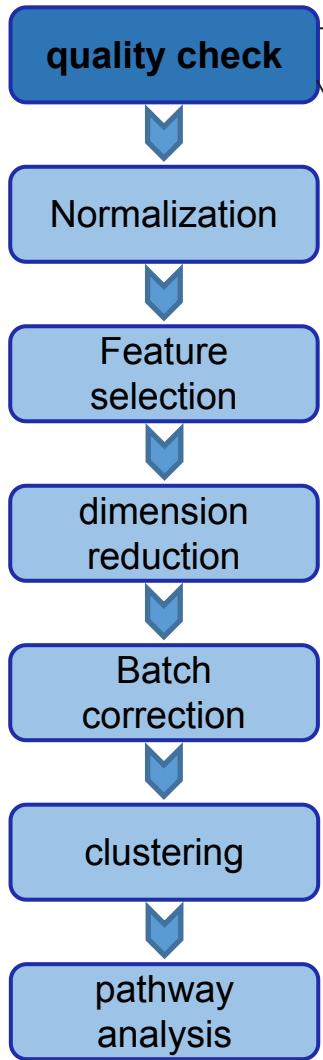
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Single cell pipeline

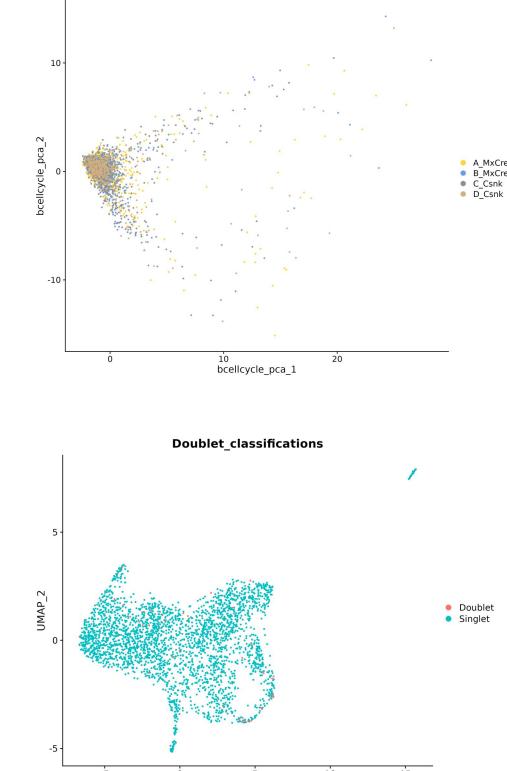
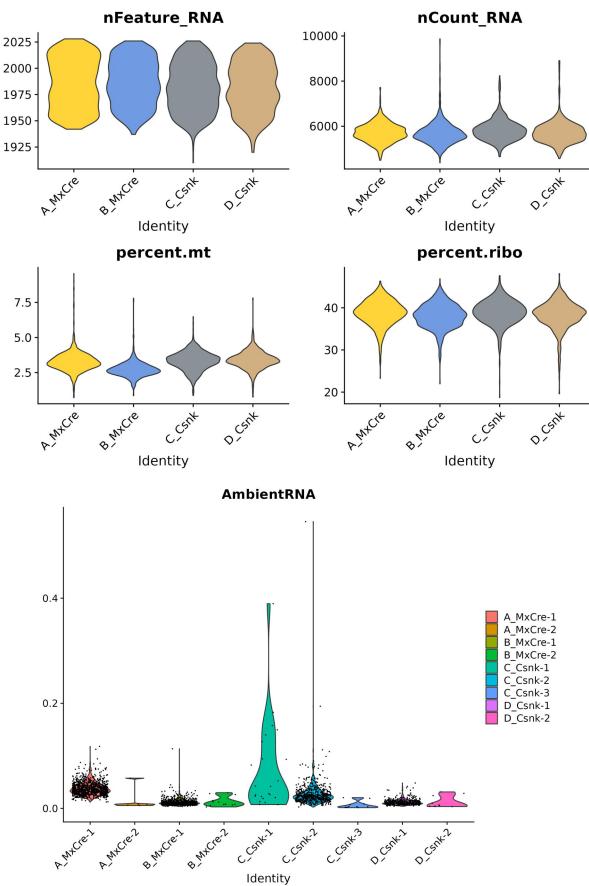
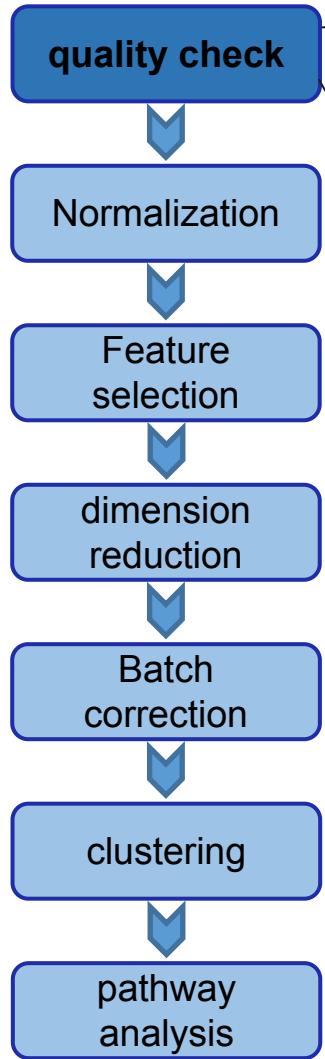


Single cell pipeline

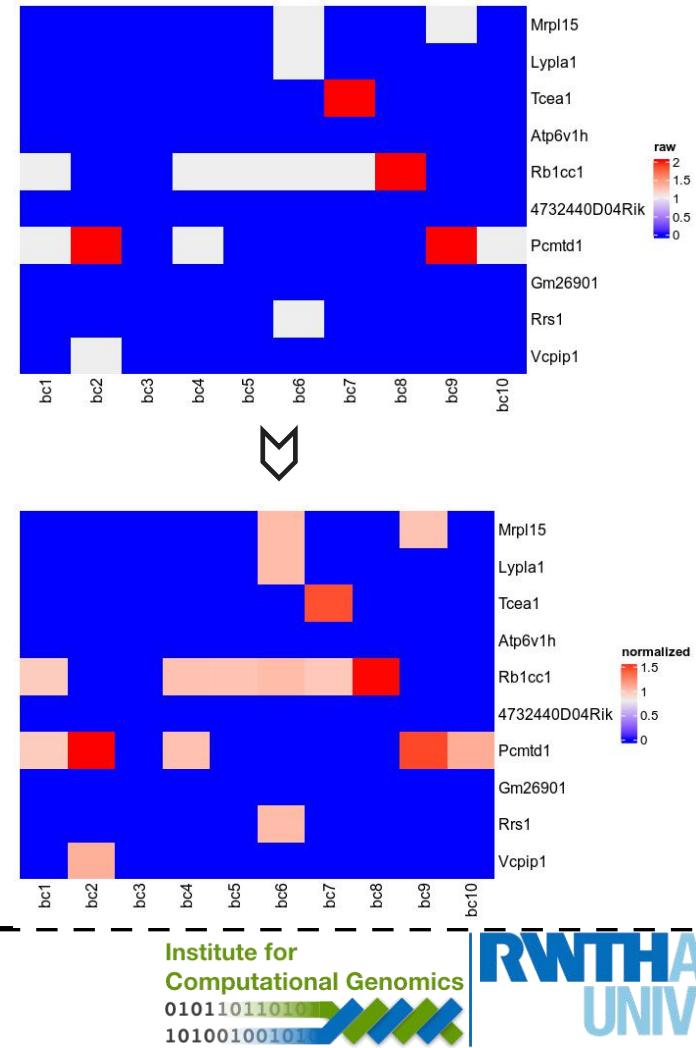
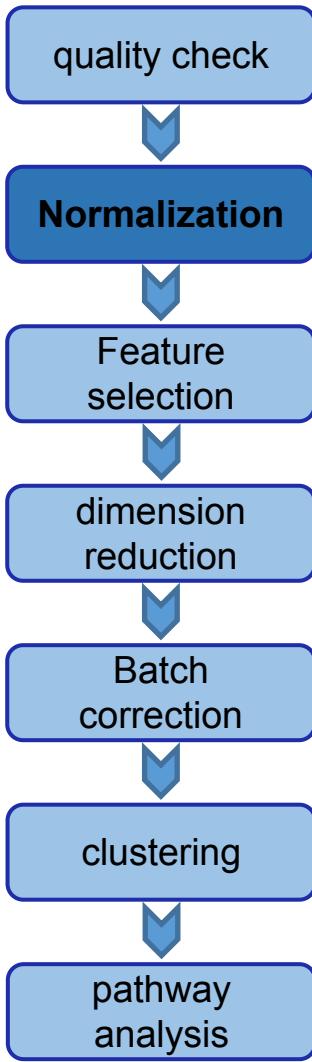


Heumos, Lukas, et al. "Best practices for single-cell analysis across modalities." *Nature Reviews Genetics* (2023): 1-23.

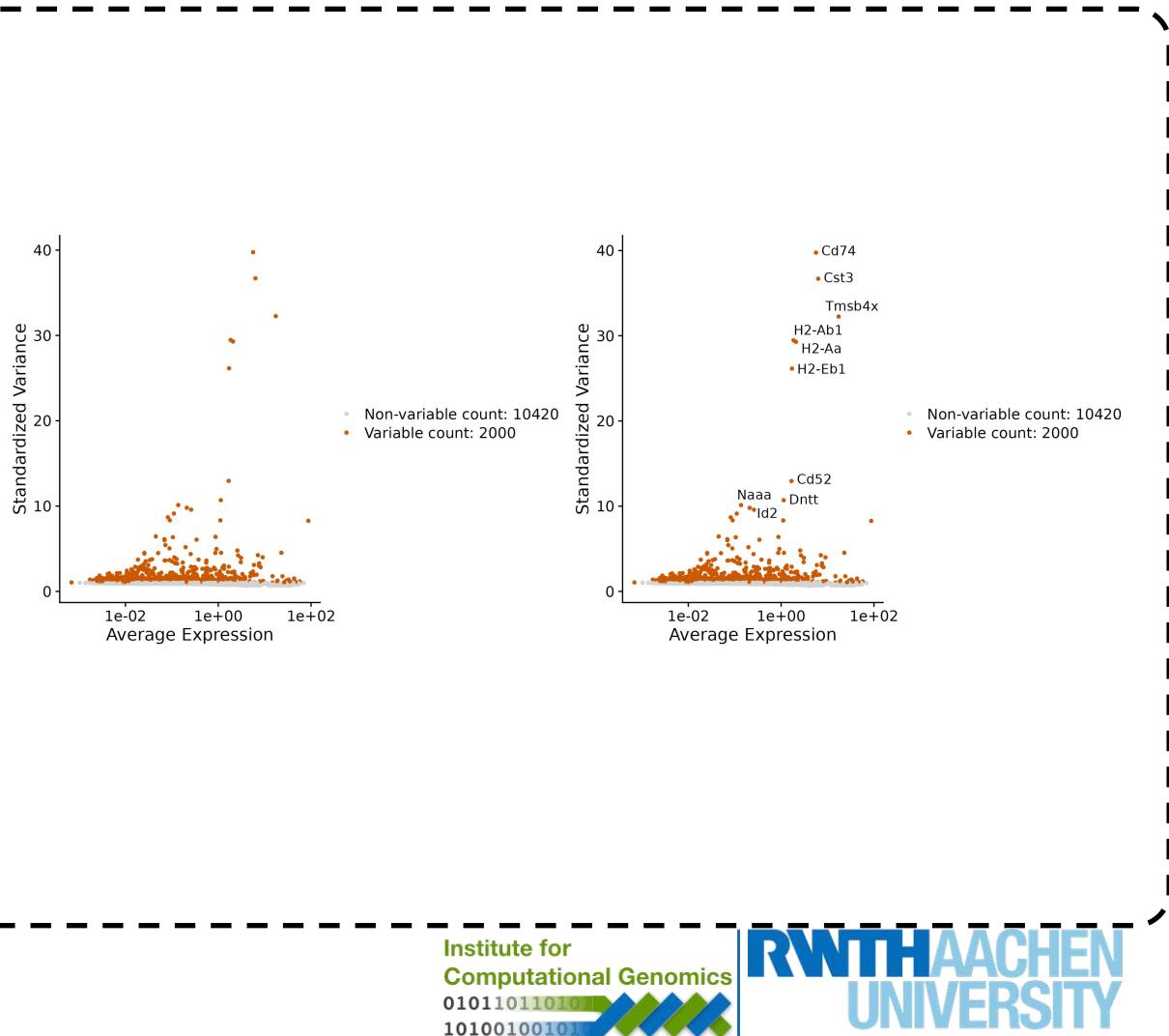
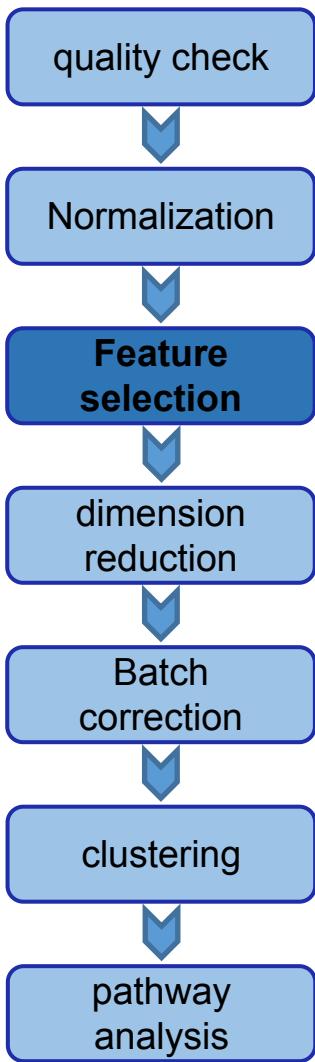
Single cell pipeline



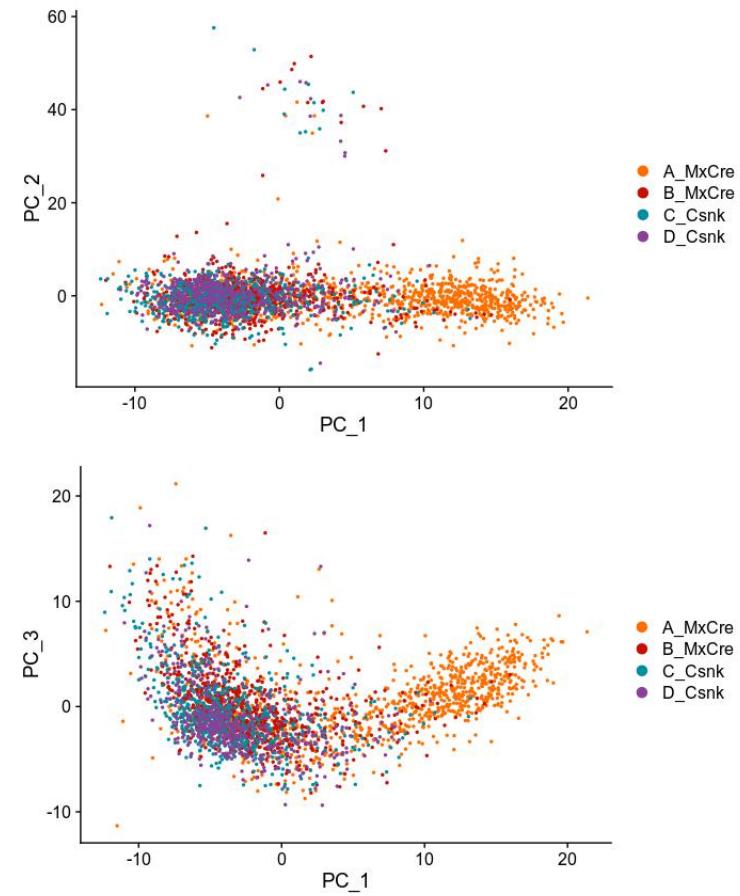
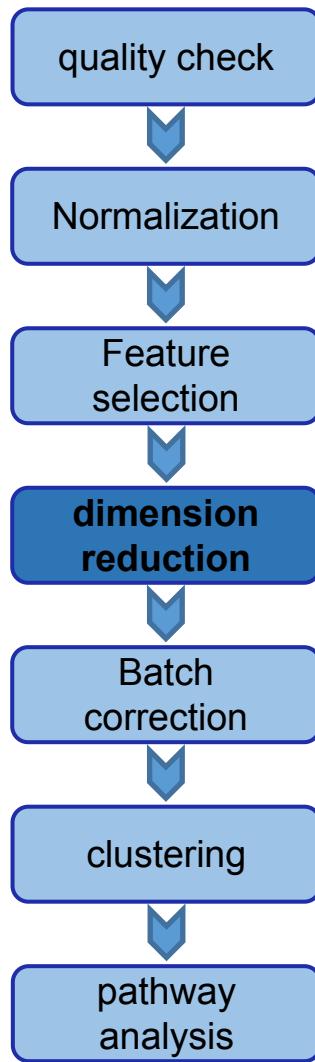
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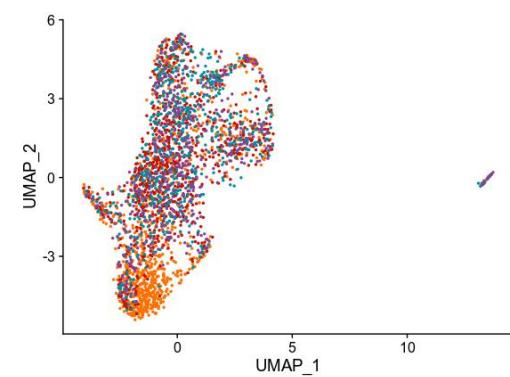
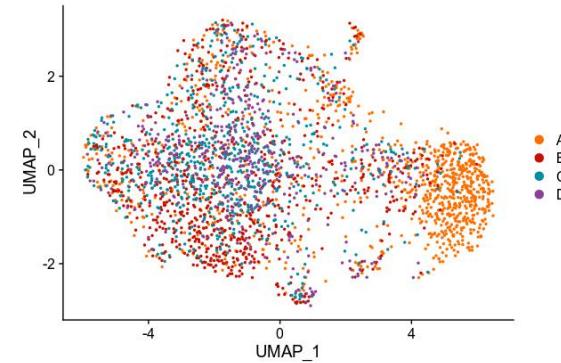
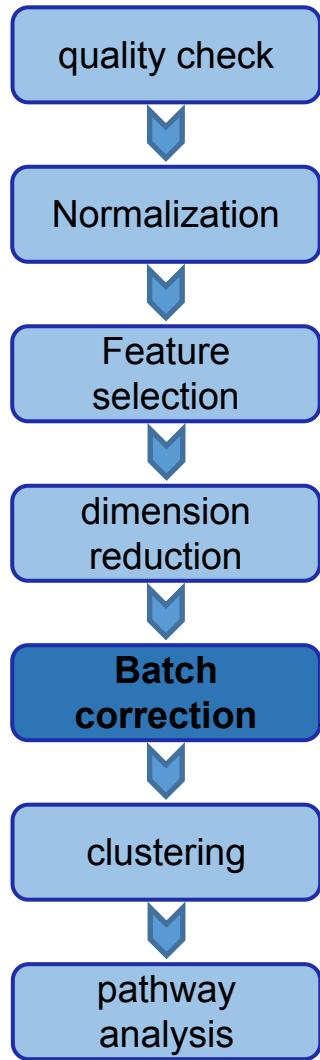
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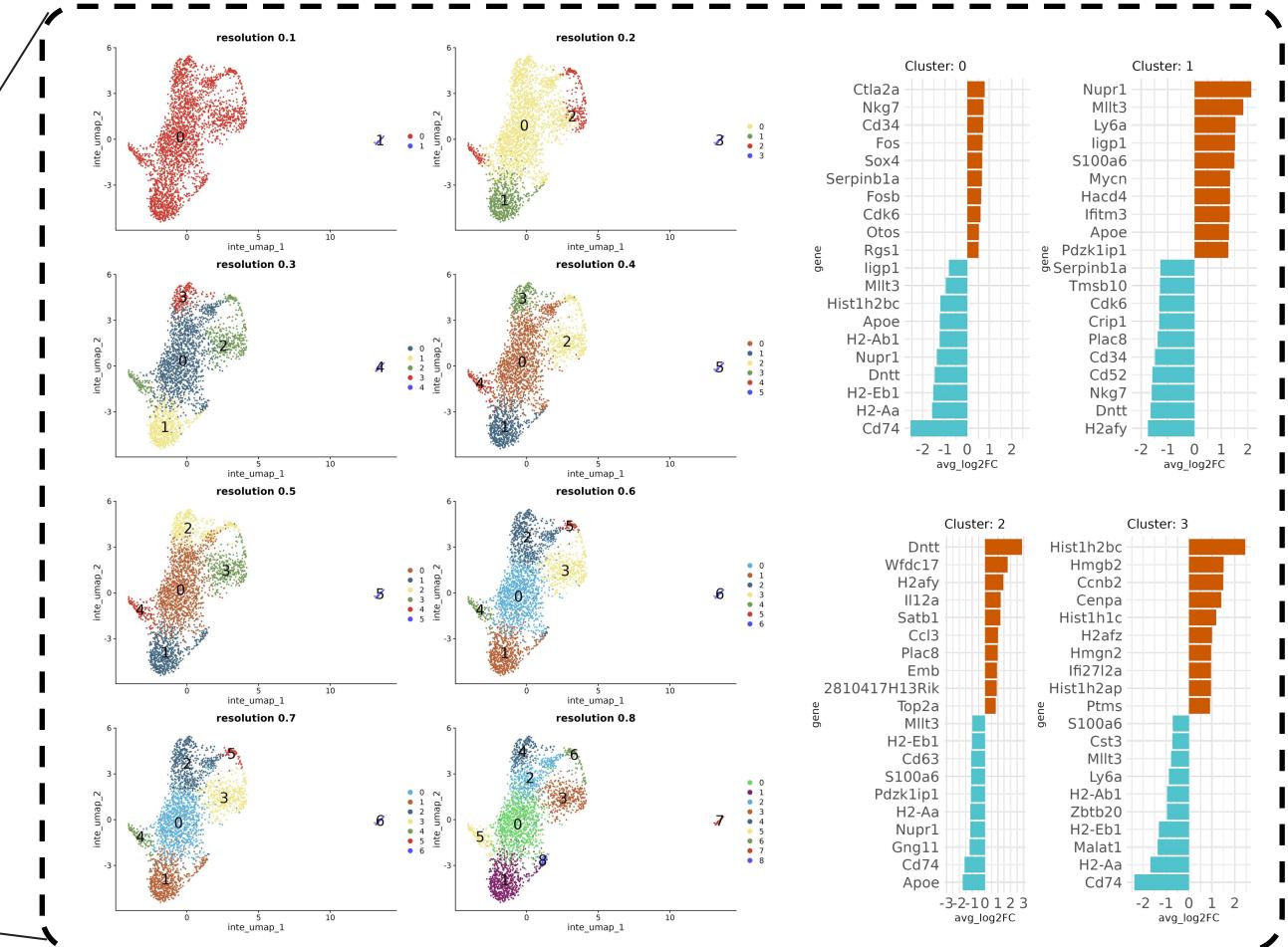
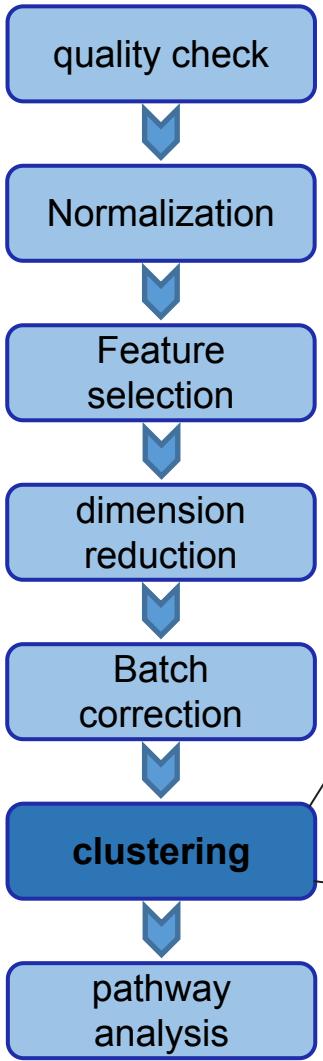
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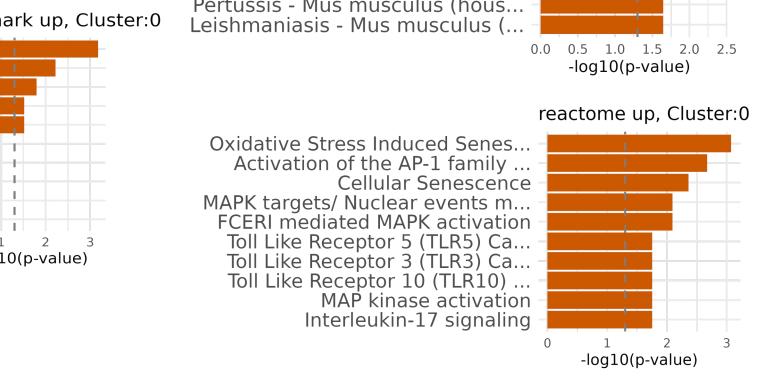
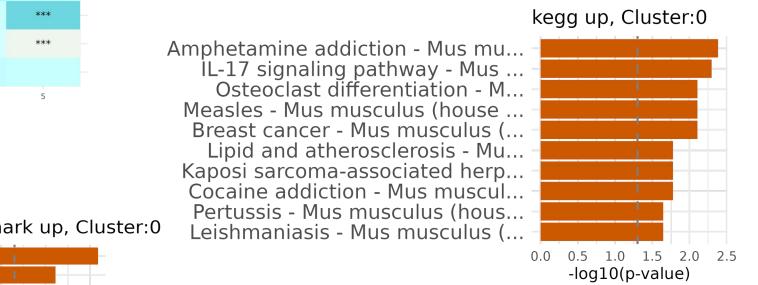
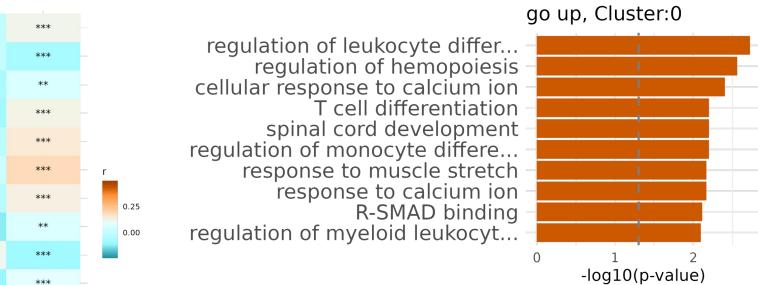
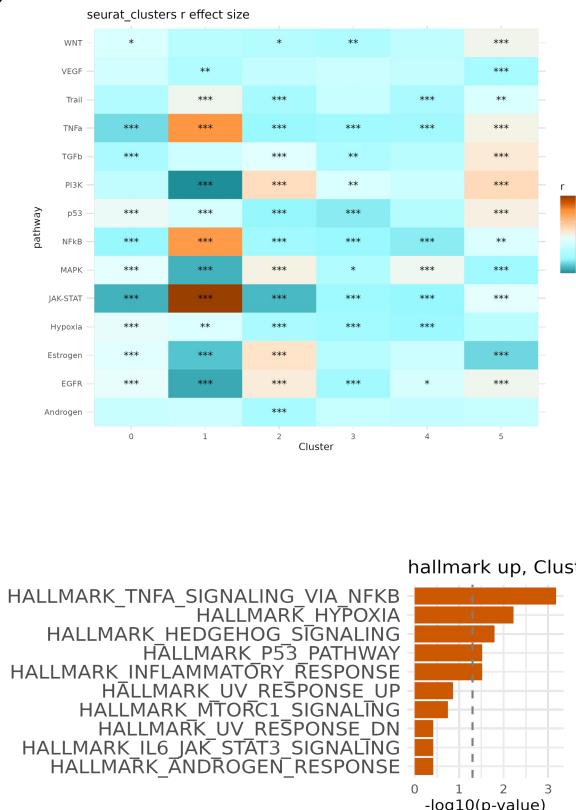
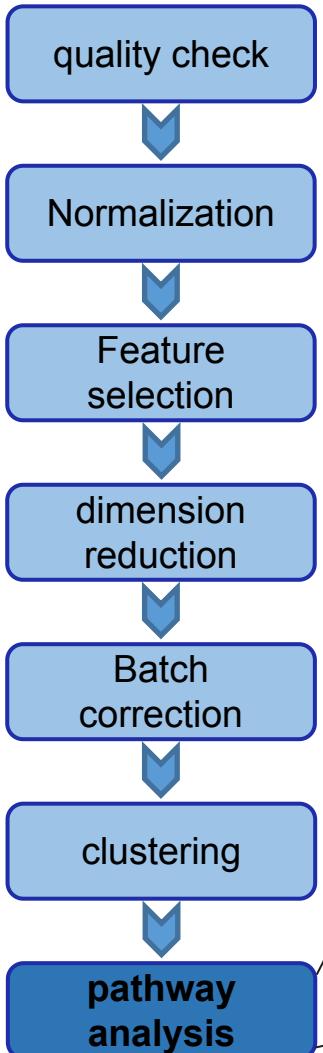
Single cell pipeline



Single cell pipeline



Single cell pipeline

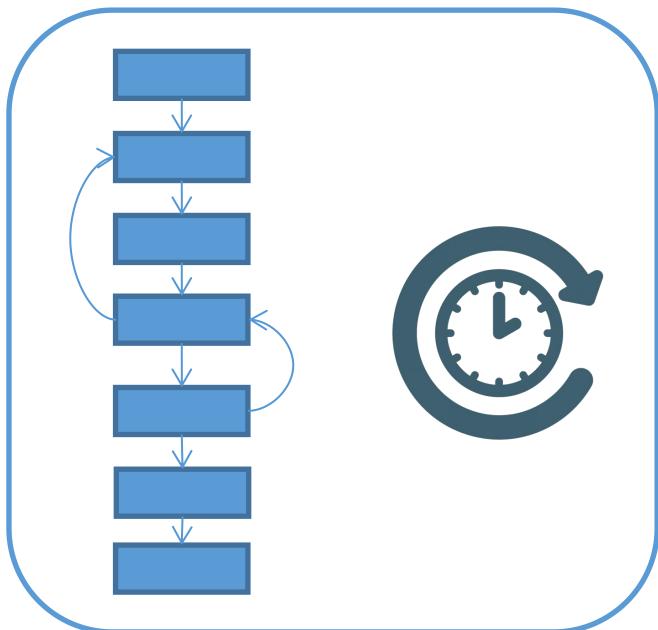


Outline

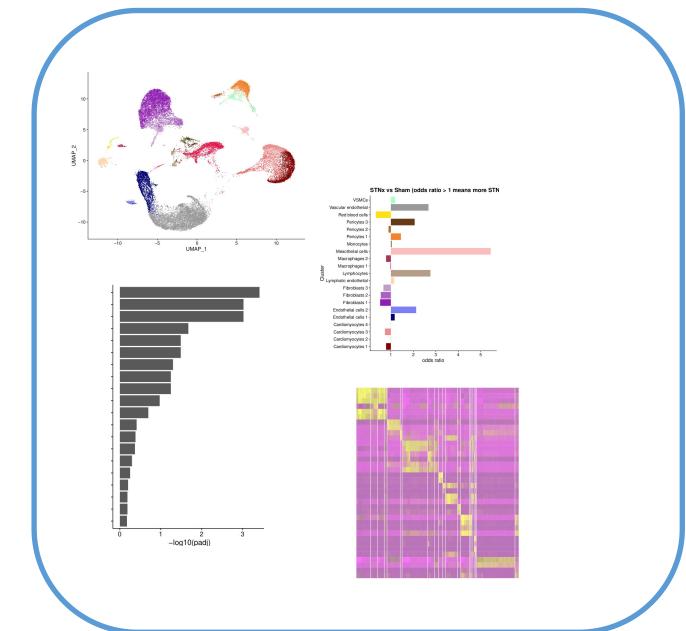
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Single cell pipeline introduction -- overview

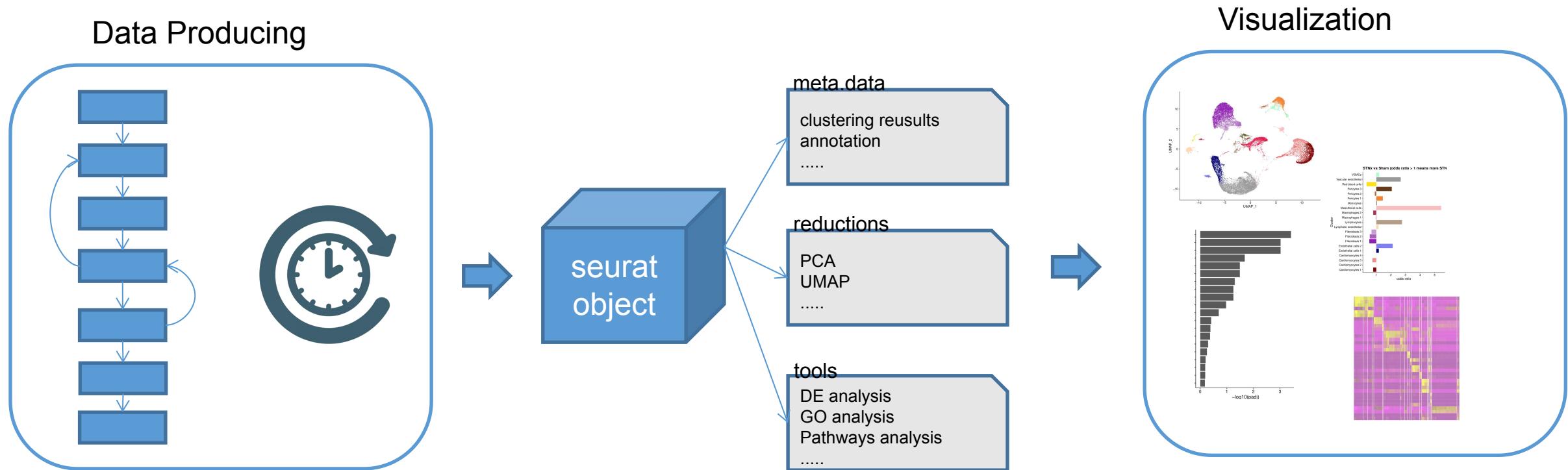
Data Producing



Visualization



Single cell pipeline introduction -- overview



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output example

Mouse Blood project Analysis Pipeline

1. Data Quality Check

- Data quality
- Data quality existing data
- Ambient RNA
- Doublet Detection

2. Batch Clustering Results

- seurat_clusters
- harmony_clusters
- Marker genes with different resolutions - Integration: seurat

3. Final Clustering Results

- clusters - Integration: seurat

4. Marker Genes & GO & pathway analysis

- External Markers
- DE & GO
 - DE & GO
 - DE-excel
 - GO-UP-excel
 - GO-DOWN-excel
- Genesets
 - Genesets
- progeny

output example

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- Hallmark
 - hallmark
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 - Hallmark-DOWN-excel

- KEGG
 - KEGG
 - KEGG-UP-excel
 - KEGG-DOWN-excel

- Reactome
 - Reactome
 - Reactome-UP-excel
 - Reactome-DOWN-excel

- Interactive UMAPs
 - Interactive UMAPs

5. Differential Expression & GO analysis(between groups)

- DE&GO pages
 - MxCre.vs.Csnk
- DE FILES
 - DE MxCre.vs.Csnk.xlsx
- GO FILES
 - GO UP MxCre.vs.Csnk.xlsx
 - GO DOWN MxCre.vs.Csnk.xlsx

6. Pathway analysis (between groups)

- Genesets
 - Genesets stage

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quick start

```
git clone https://github.com/CostaLab/scrna_seurat_pipeline.git
```

```
Rscript packages_install.R
```

```
cd scrna_seurat_pipeline
```

```
cp conf/config.R conf/config_toy.R      ## toy configuration file
```

```
cp run_example.sh run_toy.sh           ## Please edit run_toy.sh to fit your environment
```

```
sh run_toy.sh toy
```

```
cp run_viz_example.sh run_viz_toy.sh    ## Please edit run_viz_toy.sh to fit your environment
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quick start

- Mainly split **execution plan** to 3 main step
 - scrna_phase_preprocess
 - scrna_phase_clustering
 - scrna_phase_comparing

quick start

- Mainly split **execution plan** to 3 main step
 - **scrna_phase_preprocess**
 - Ambient RNA detection
 - doublets detection
 - **Filteration**
 - etc.
 - **scrna_phase_clustering**
 - **scrna_phase_comparing**

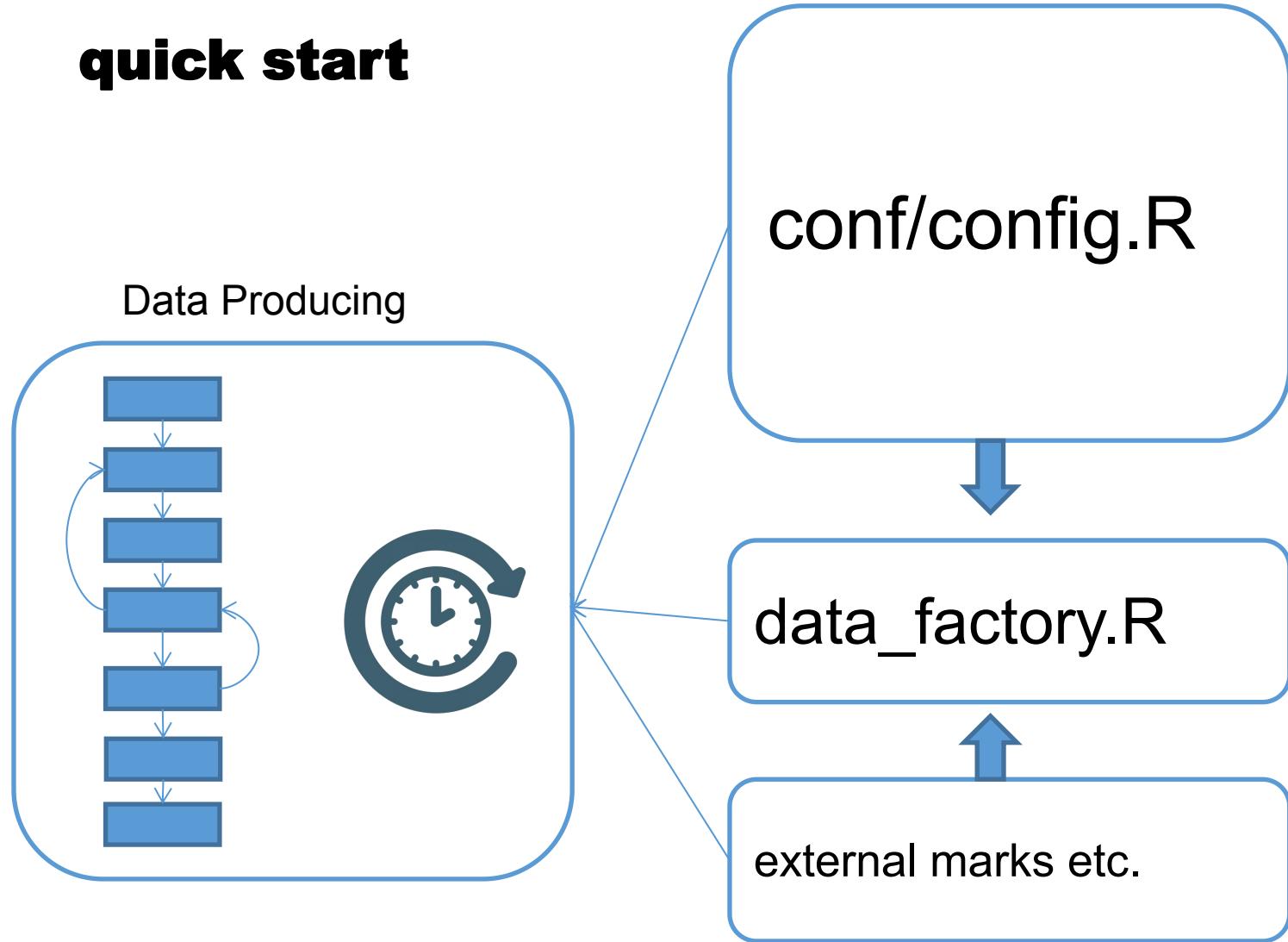
quick start

- Mainly split **execution plan** to 3 main step
 - scrna_phase_preprocess
 - **scrna_phase_clustering**
 - Batch correction
 - Clustering
 - MCA, HCL annotation
 - etc.
 - scrna_phase_comparing

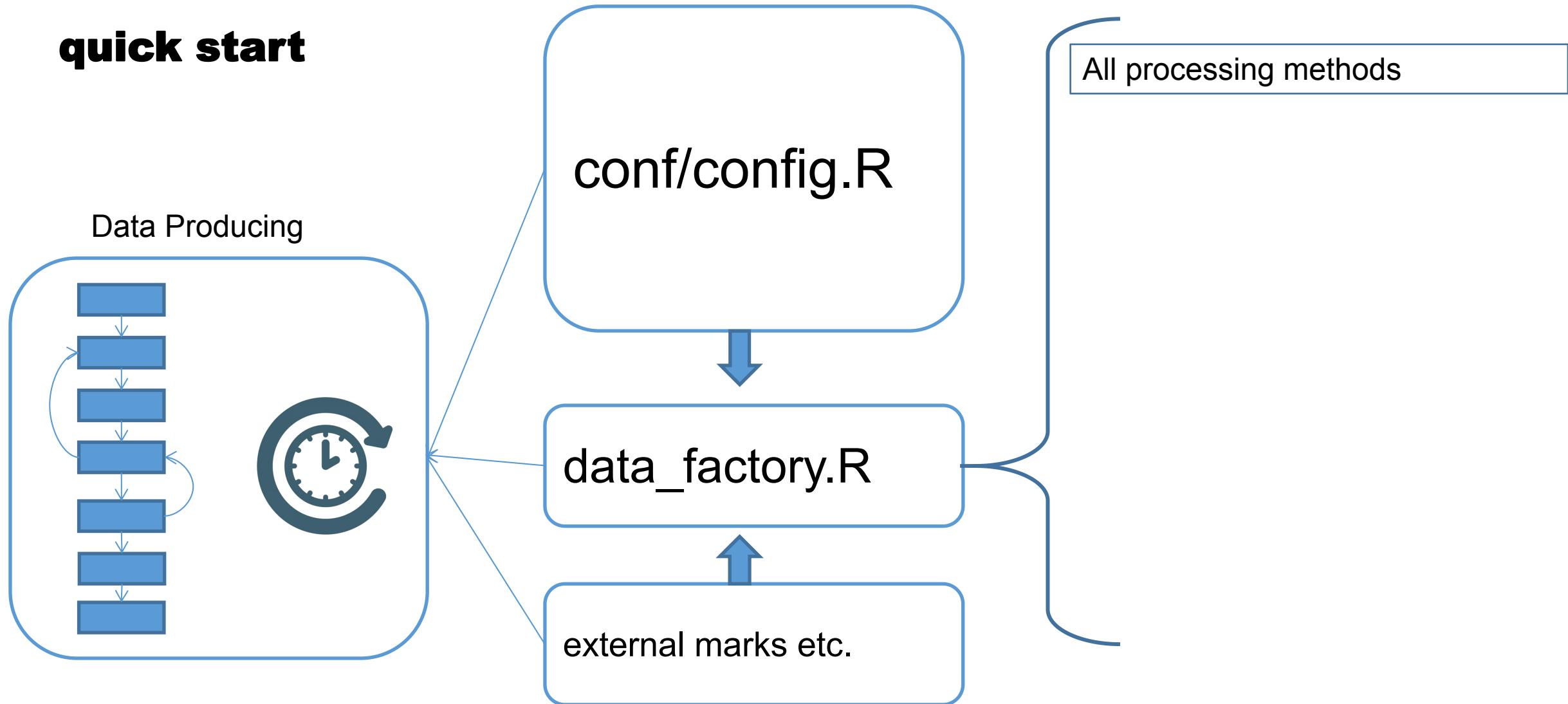
quick start

- Mainly split **execution plan** to 3 main step
 - scrna_phase_preprocess
 - scrna_phase_clustering
 - **scrna_phase_comparing**
 - fishertest etc.
 - progeny analysis
 - DE analysis
 - GO, KEGG, Reactome, HALLMARK
 - etc.

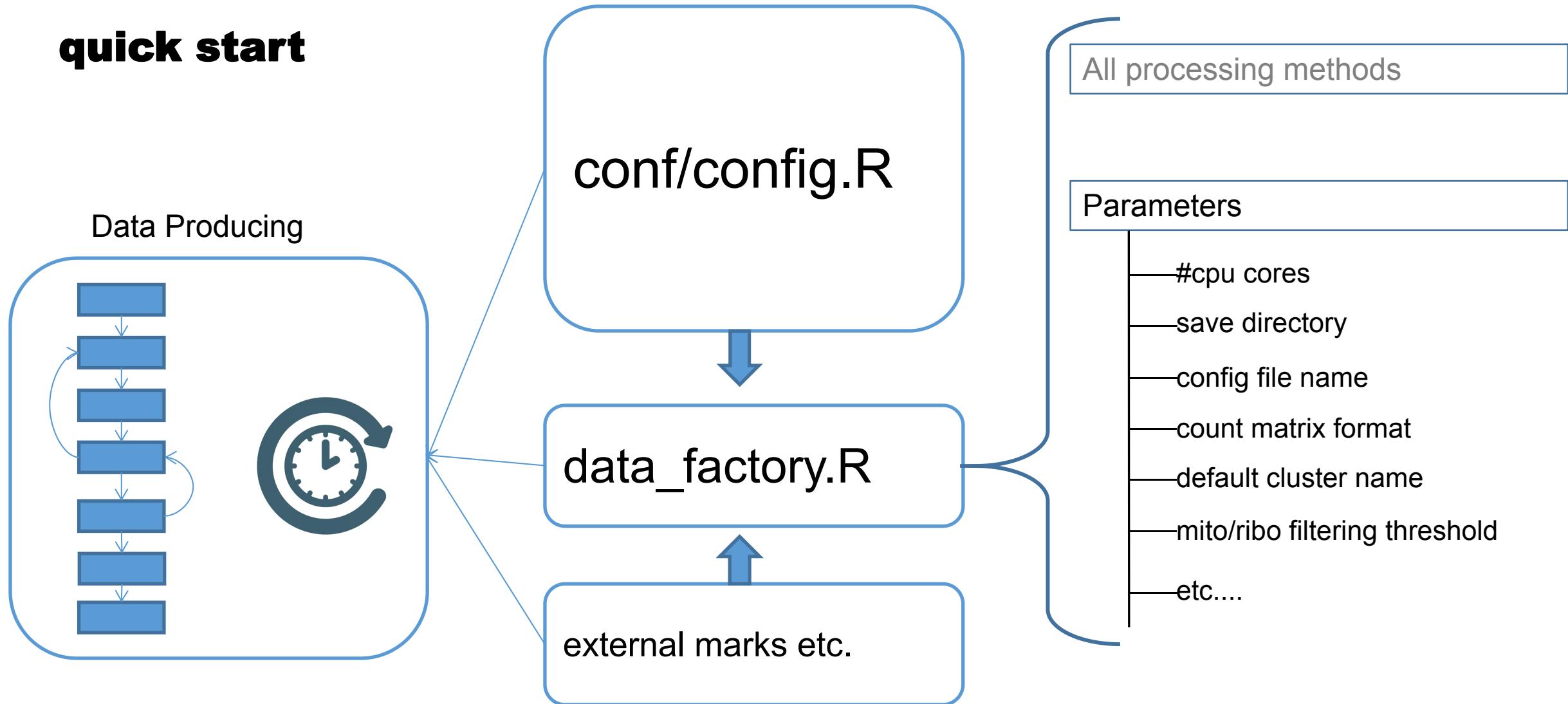
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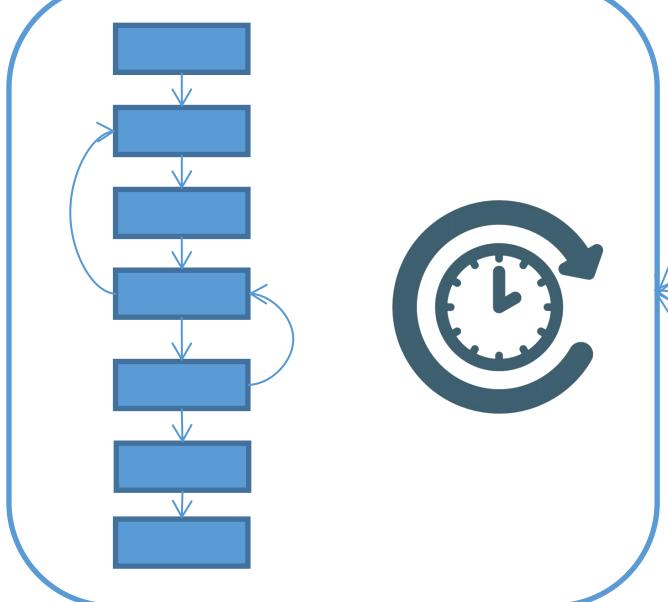


quick start



quick start

Data Producing



run_toy.sh

All processing methods

Parameters

- #cpu cores
- save directory
- config file name
- count matrix format
- default cluster name
- mito/ribo filtering threshold
- etc....

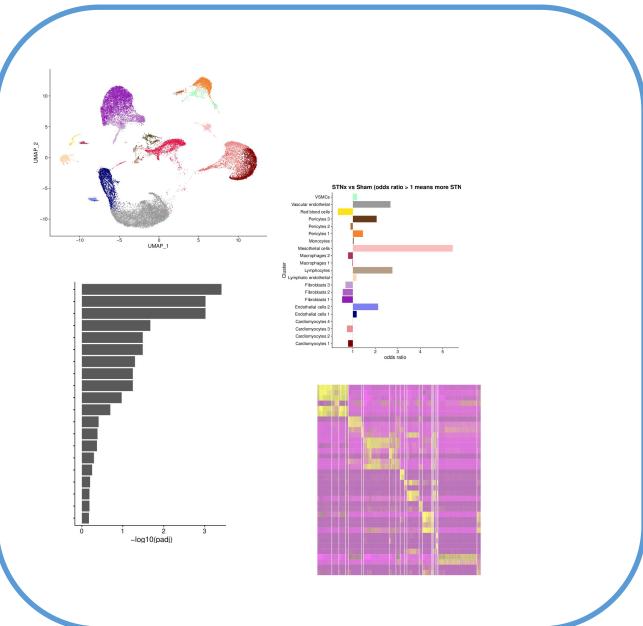
quick start

- conf/config.R
- excels generated
- Seurat Objects

run_viz_toy.sh

- 1_quality_report.Rmd
- 2_clustering.Rmd
- 2_clusters_DEs.Rmd
- 3_DE_GO-analysis.Rmd
- 3_external_markers.Rmd
- make_report.R
- singleton_clustering.Rmd
- template/
 - DE-GO-vs.template
 - Genesets-vs.template
 - index.template

Visualization



report

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```
vim config.R 99x58
### -----Initial info-----
PROJECT = "Mouse Blood project" ## set project name
ORGAN = 'Blood'                 #For external annotation. Options: see below(External Organs)
SPECIES = "Mouse"                #For external annotation. Options: Human, Mouse
MCA_NAME = "Bone-Marrow" #For MCA annotation.      Options: check http://bis.zju.edu.cn/M
HCL_NAME = "Adult-Bone-Marrow-CD34P" #For HCL annotation.

# filtering params when create seurat object
MINCELLS = 5
MINGENES = 50

INTEGRATION_OPTION = "seurat" ### or harmony

### ----- Data SRC -----
ANNOTATION_EXTERNAL_FILE = "external/Human_and_mouse_cell_markers-Markers.tsv"

## If genesets you need are not included, please attach your geneset to the gmt.gz file.
MSigDB_GENESET_HUMAN_GMT_FILE = "external/Human_msigdb.v7.2.symbols.gmt.gz"

data_src = c(
  A_MxCre     = "data/A_MxCre",
  B_MxCre     = "data/B_MxCre",
  C_Csnk      = "data/C_Csnk",
  D_Csnk      = "data/D_Csnk"
)

##----- SET REPLICATE GROUP -----
stage_lst = c(
  A_MxCre     = "MxCre",
  B_MxCre     = "MxCre",
  C_Csnk      = "Csnk",
  D_Csnk      = "Csnk"
)
```

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```
## 0. omit,      1. calc & save,      2. load
conf = c(
  scrna_phase_preprocess      = 1, ## qual
  scrna_phase_clustering      = 1, ## integ
  scrna_phase_comparing       = 1, ## DE GO
  scrna_cluster_annotation    = 0, ## Annot
  scrna_clusterwise_xcell     = 2, ## remov
  scrna_del_mitogenes         = 0, ## !!!DA
  scrna_merge_clusters        = 0, ## merge
  scrna_remove_clusters       = 0, ## remov
  scrna_remove_recluster      = 0) ## remov
```

Outline

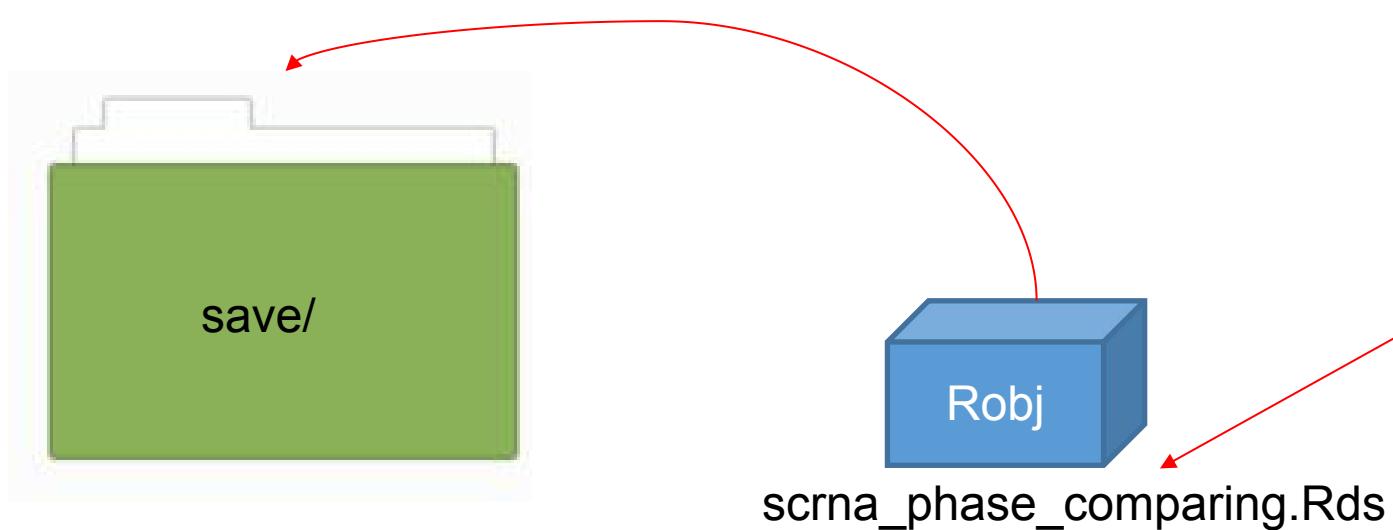
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frequently asked questions

- Is it possible to do further analysis given an existed Seurat Object?

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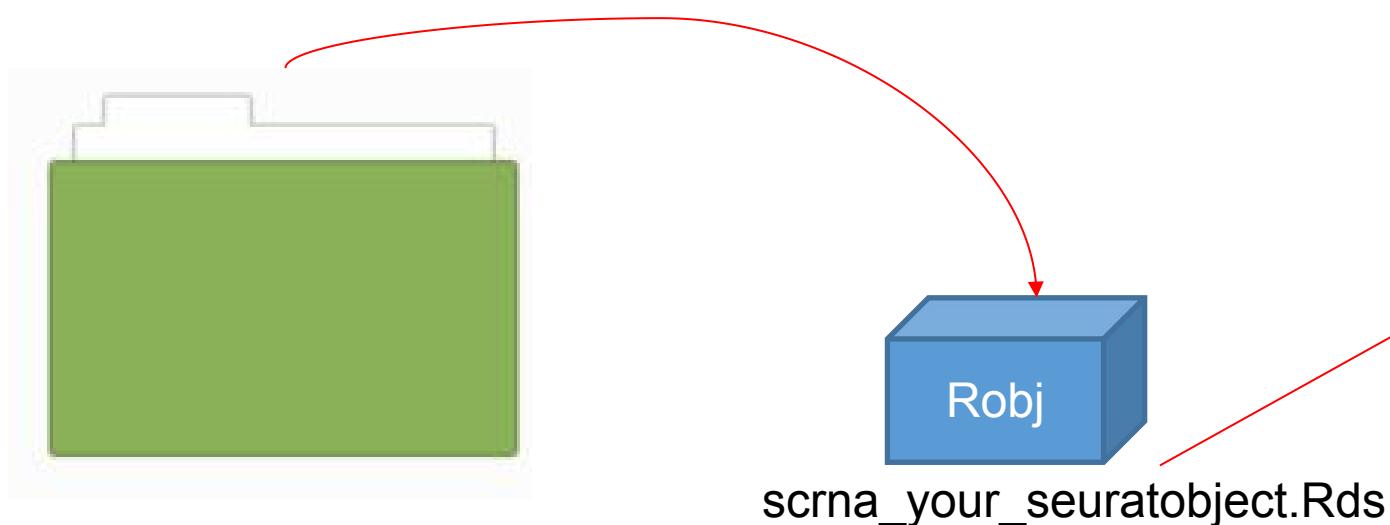
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conf = c(
  scrna_phase_preprocess = 1, ## quali
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```
## 0. omit,      1. calc & save,      2. load
conf = c(
  scrna_phase_preprocess = 0, ## qua
  scrna_phase_clustering = 0, ## ini
  scrna_your_seuratobject = 2, ## DE
  scrna_phase_clustering = 1, ## ini
  scrna_phase_comparing = 1, ## DE
  scrna_cluster_annotation = 0, ## An
  scrna_clusterwise_xcell = 0, ## ren
  scrna_del_mitogenes = 0, ## !!
  scrna_merge_clusters = 0, ## me
  scrna_remove_clusters = 0, ## ren
  scrna_remove_recluster = 0) ## ren
```

frequently asked questions

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- Why I failed to load the RObject in the save folder?

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 - ```
source("R/save_load_helper.R")
scrna <- load_object("scrna_phase_comparing")
```

## frequently asked questions

- Is it possible to do further analysis given an Seurat Object?
- Why I failed to load the RObject in the save folder?
- Why not using nextflow or snakemake?

# frequently asked questions

- Is it possible to do further analysis given an Seurat Object?
- Why I failed to load the RObject in the save folder?
- Why not using nextflow or snakemake?
  - Too many I/O tasks
  - More flexible to do the analysis
  - Nextflow produces too many files leading the RWTH-HPC overloaded

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  - Too many I/O tasks
  - More flexible to do the analysis
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```
vim phase.ini 78x48
1 [phase_preprocess] = 1
2 scrna_rawdata = 1
3 scrna_ambient_rna = 1
4 scrna_filter = 1
5 scrna_doublet_proportions = 1
6 scrna_preprocess = 1
7 scrna_cellcycle = 1
8 scrna_cycleRegressOut = 1
9 scrna_regressOut = 1
10
11
12
13 [phase_clustering] = 1
14 scrna_integration_harmony = 1
15 scrna_integration_seurat = 1
16 scrna_batchclustering = 1
17 scrna_batch_markergenes = 1
18 scrna_clustering = 1
19 scrna_fishertest_inte_clusters = 1
20 scrna_fishertest_clusters = 1
21 scrna_proptest_clusters = 1
22 scrna_MCAannotate = 1
23 scrna_ExternalAnnotation = 1
24 scrna_HCLannotate = 1
25 scrna_MAGIC = 1
```

## frequently asked questions

- Is it possible to do further analysis given an Seurat Object?
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- Why not using nextflow or snakemake?
- The pipeline require too much memory

# frequently asked questions

- Is it possible to do further analysis given an Seurat Object?
- Why I failed to load the RObject in the save folder?
- Why not using nextflow or snakemake?
- The pipeline require too much memory
  - Please set `--allinone=FALSE` in `run_toy.sh`

# **useful links**

- **source code**
  - [https://github.com/CostaLab/scrna\\_seurat\\_pipeline](https://github.com/CostaLab/scrna_seurat_pipeline)
- **tutorial**
  - [https://costalab.github.io/scrna\\_seurat\\_pipeline/test/tutorial.html#run-data-producing](https://costalab.github.io/scrna_seurat_pipeline/test/tutorial.html#run-data-producing)
- **Link**
  - [https://github.com/CostaLab/scrna\\_seurat\\_pipeline/blob/master/docs/A\\_comprehensive\\_singlecell\\_RNA\\_analysis\\_pipeline.pdf](https://github.com/CostaLab/scrna_seurat_pipeline/blob/master/docs/A_comprehensive_singlecell_RNA_analysis_pipeline.pdf)

# Thanks

## Q&A