SinCellTE 2022

Practice : Primary Analysis

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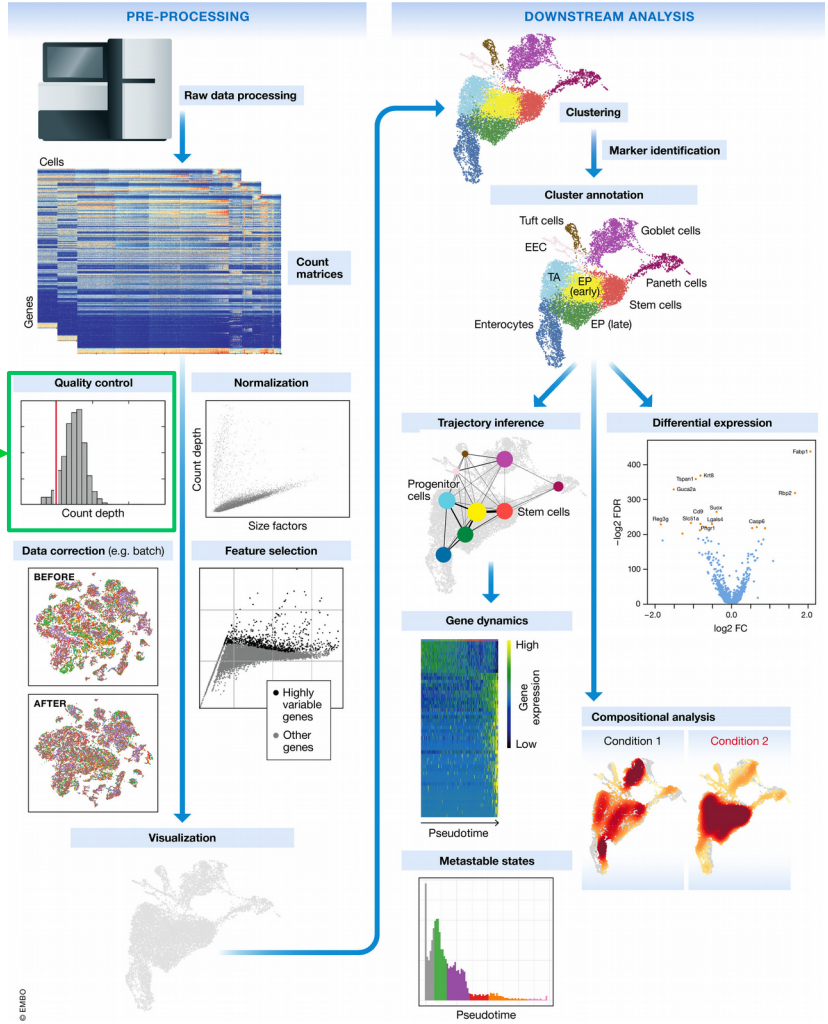
Main steps of single cell data processing

This practical session



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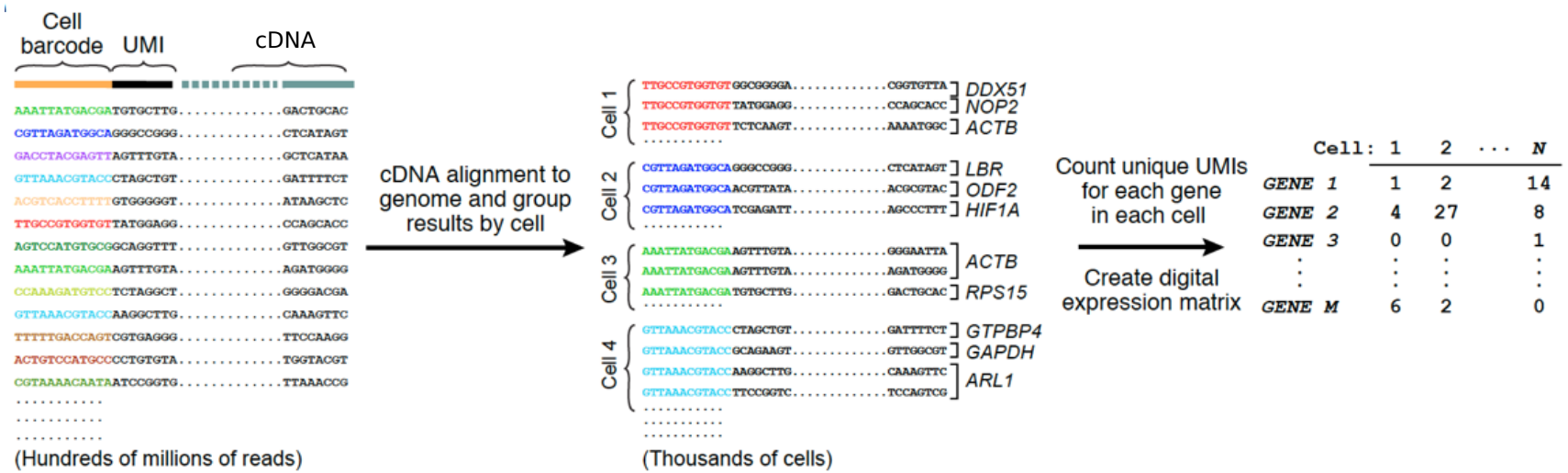
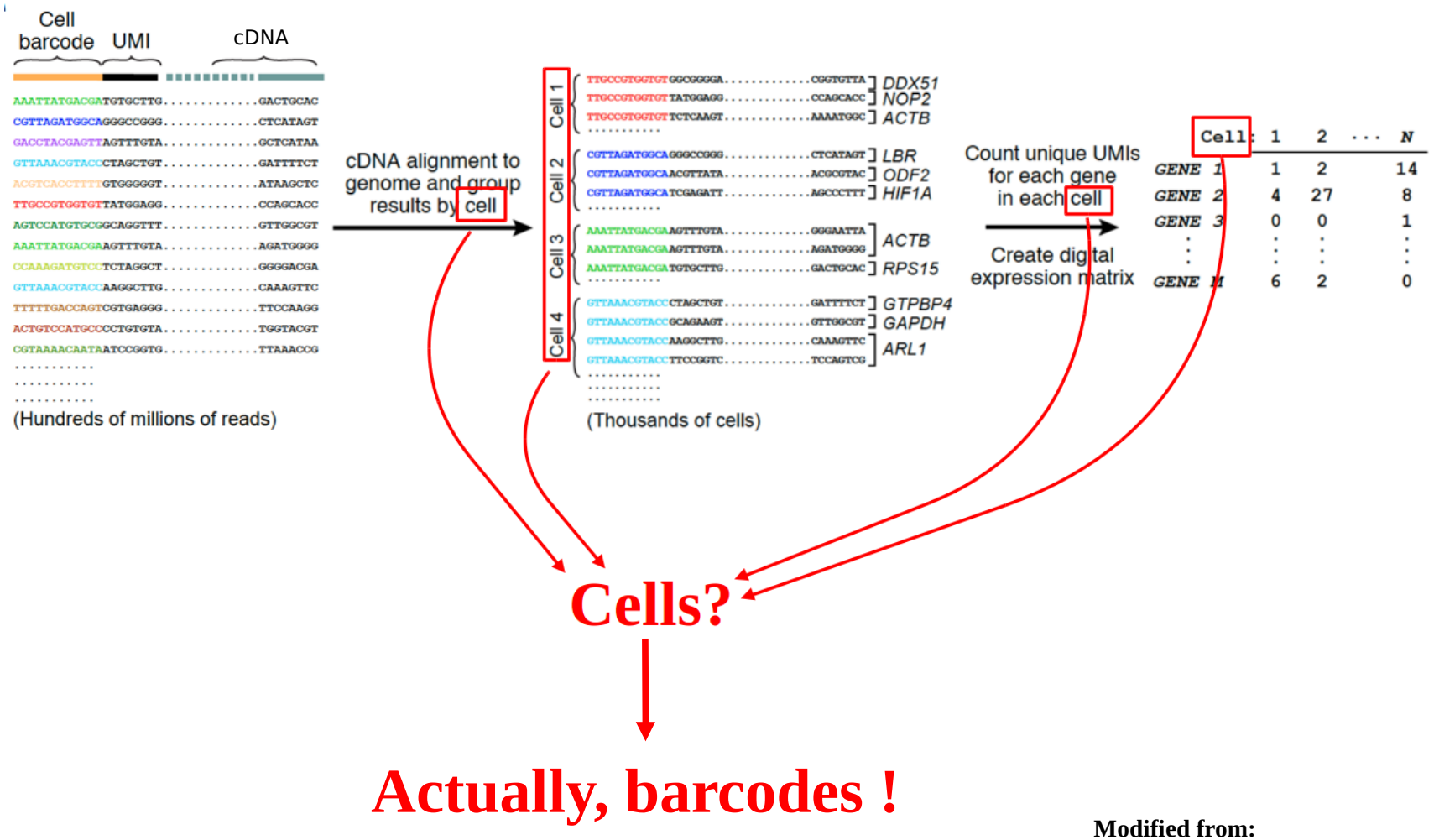
Theoretical Part

Alignment



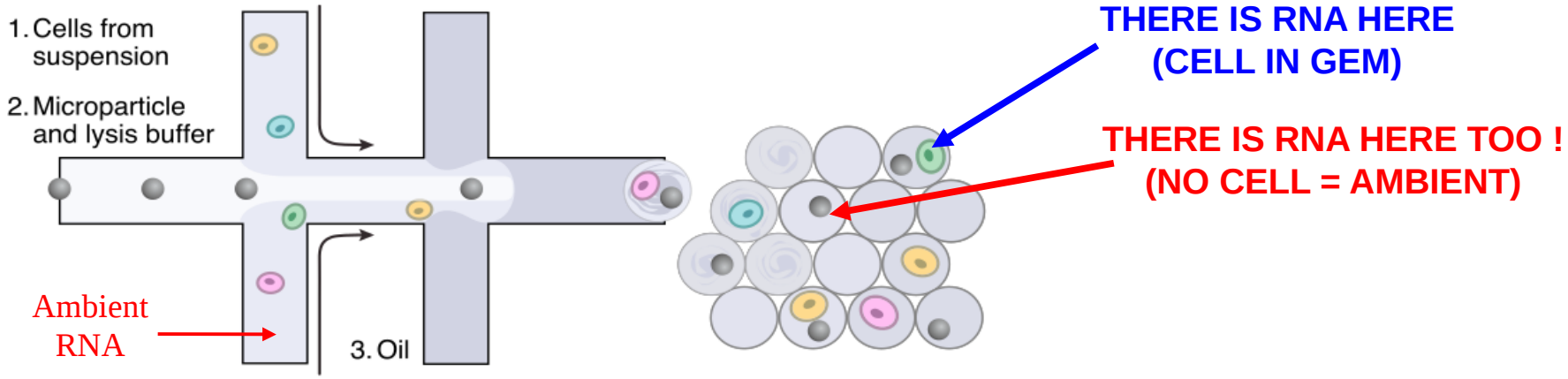
Modified from:
http://mccarrolllab.org/dropseq/

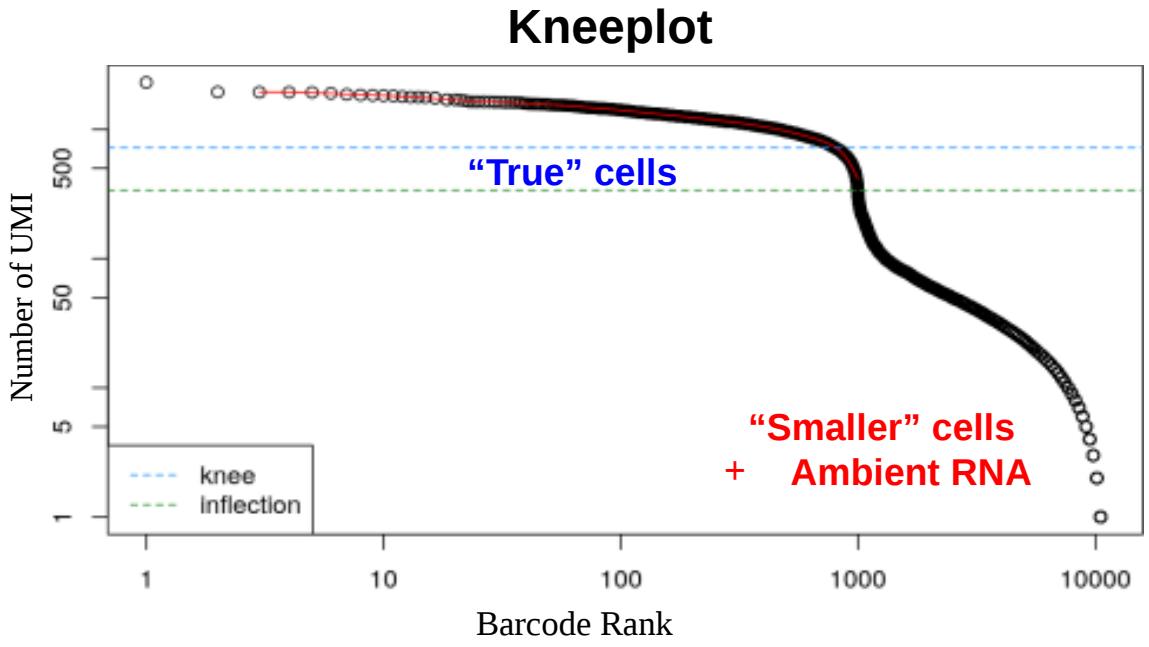
Alignment



Modified from:
http://mccarrolllab.org/dropseq/

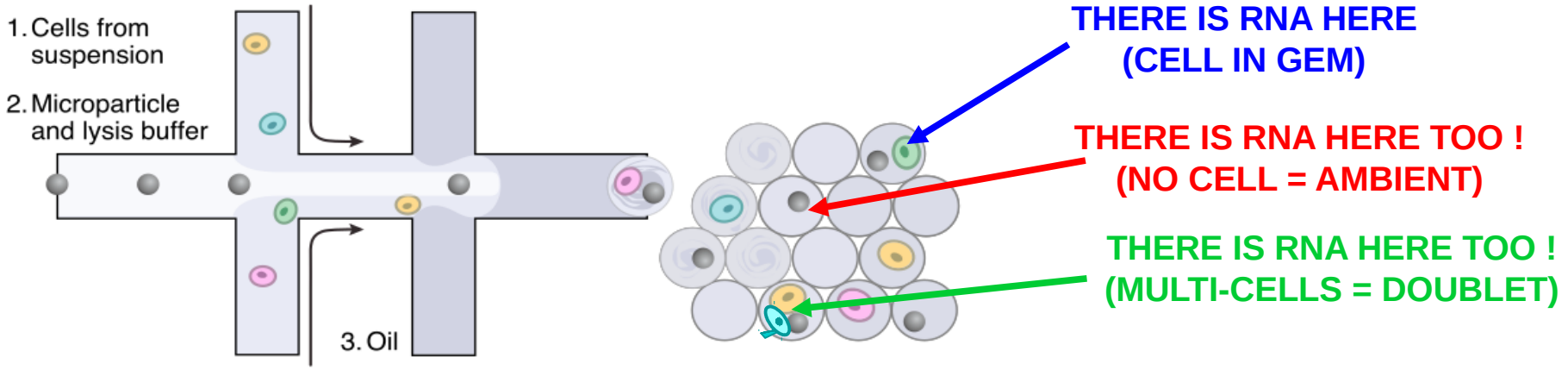
Filtering droplets: empty droplets

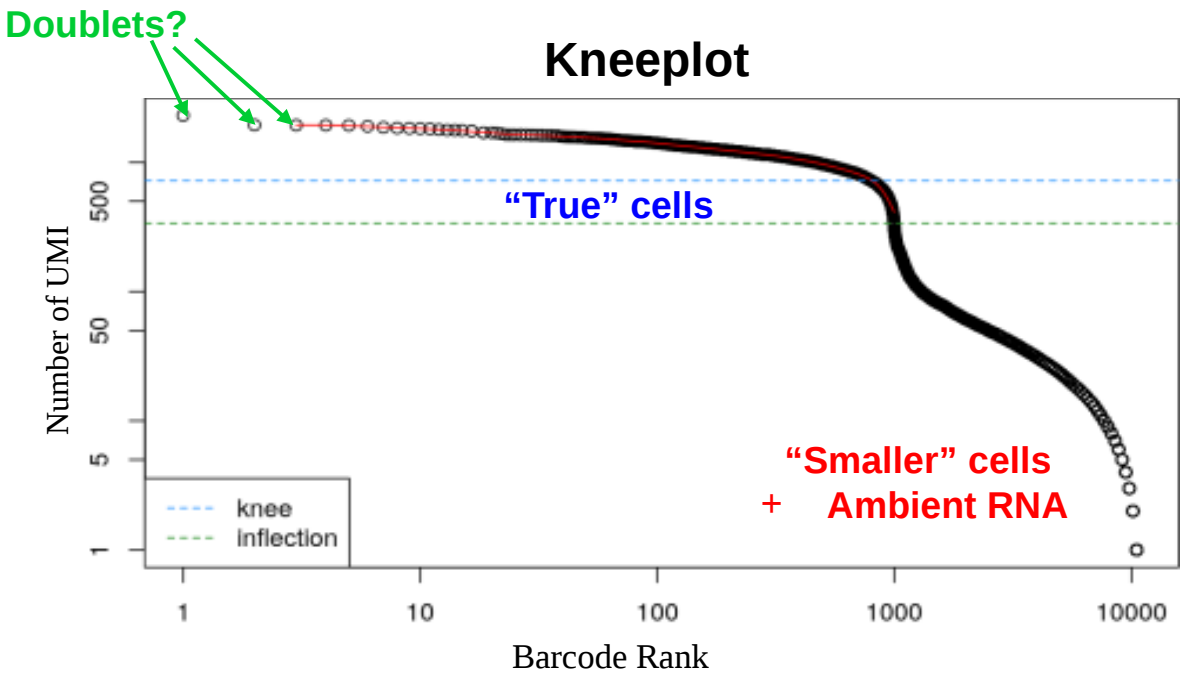




- Viability = 70%
  → 30% dead cells
  → ambient RNA
  → noise in empty droplet
  + noise in droplet with cell
- Package R: EmptyDrops
  Raw thresholds

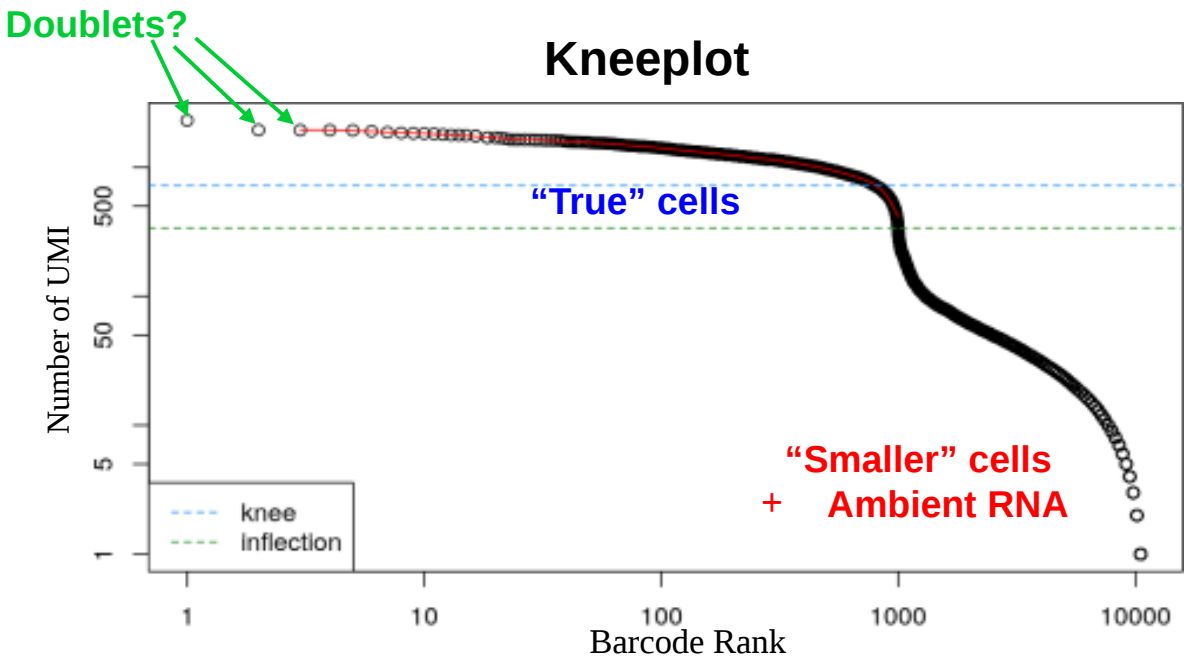
Filtering droplets: doublets or multiplets

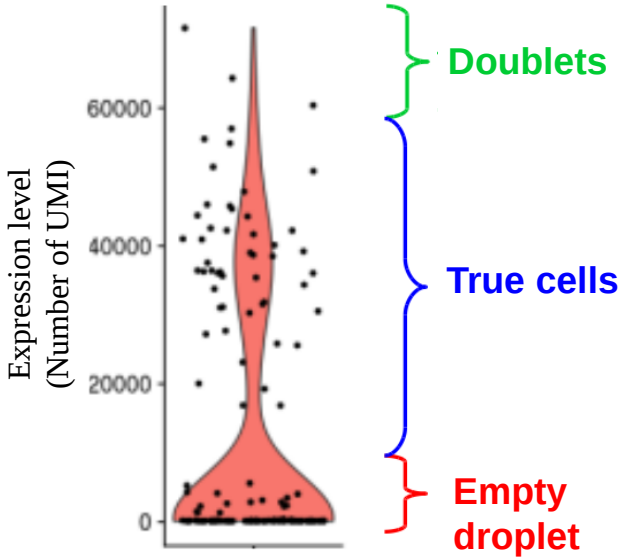


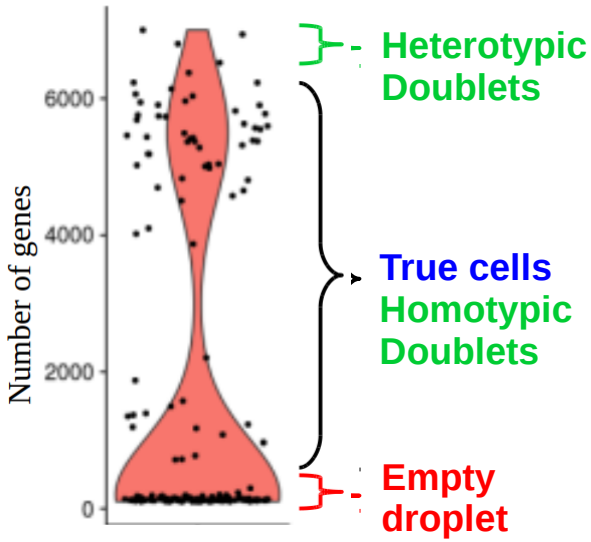


- Doublet types:
  - Homotypic: same cell type in droplet
  - Heterotypic: different cell types in droplet
- Doublet rate:
  - 1% for 1000 cells
  - 5% for 10 000 cells

Filtering droplets: doublets or multiplets







What is an expressed gene ?

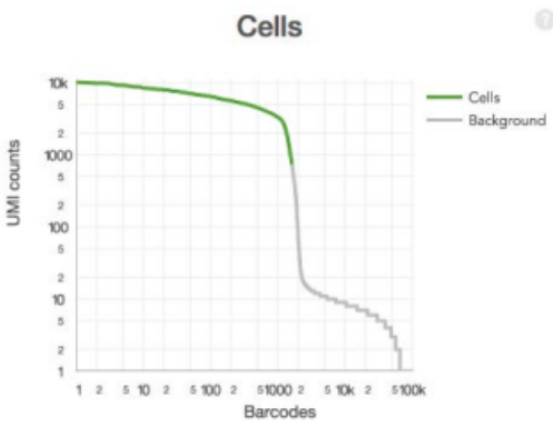
- Minimum amount of UMIs ?
  (Be careful ! Droplet => low depth!)
- Minimum amount of expressing cells ?
- Minimum gene expression level ?

How to select a threshold ?

- Kept genes : expressed in >= 5 cells
- Kept cells : counts > 0 for at least 200 genes
- Prior knowledge : expected amount of cells in the sample

Kneeplot: Diagnosis

Typical Sample Profile

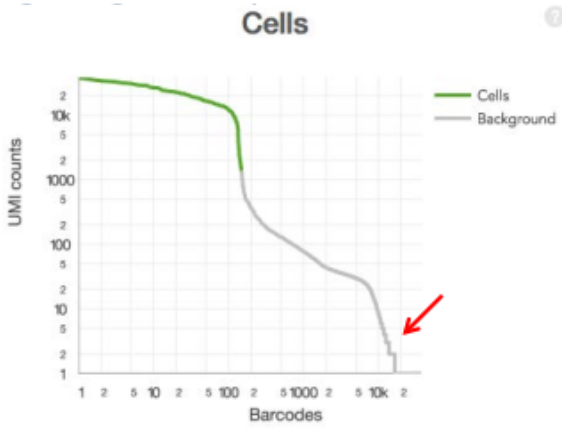


Defined cliff and knee

Metric	Value
Barcodes	> 90,000
Cell Barcodes	> 1,000
UMIs	> 10,000

Good!

Low Barcode Counts



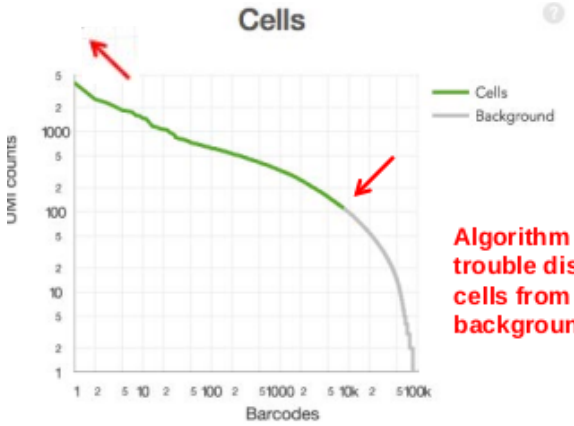
Low number of barcodes detected

Metric	Value
Barcodes	~ 15,000
Cell Barcodes	> 100
UMIs	> 10,000

Bad!

Depth is too low : although no sequencing of ambient RNA, there is almost no sequencing of genes neither !

Loss of Single Cell Behaviour



Algorithm has trouble discerning cells from the background

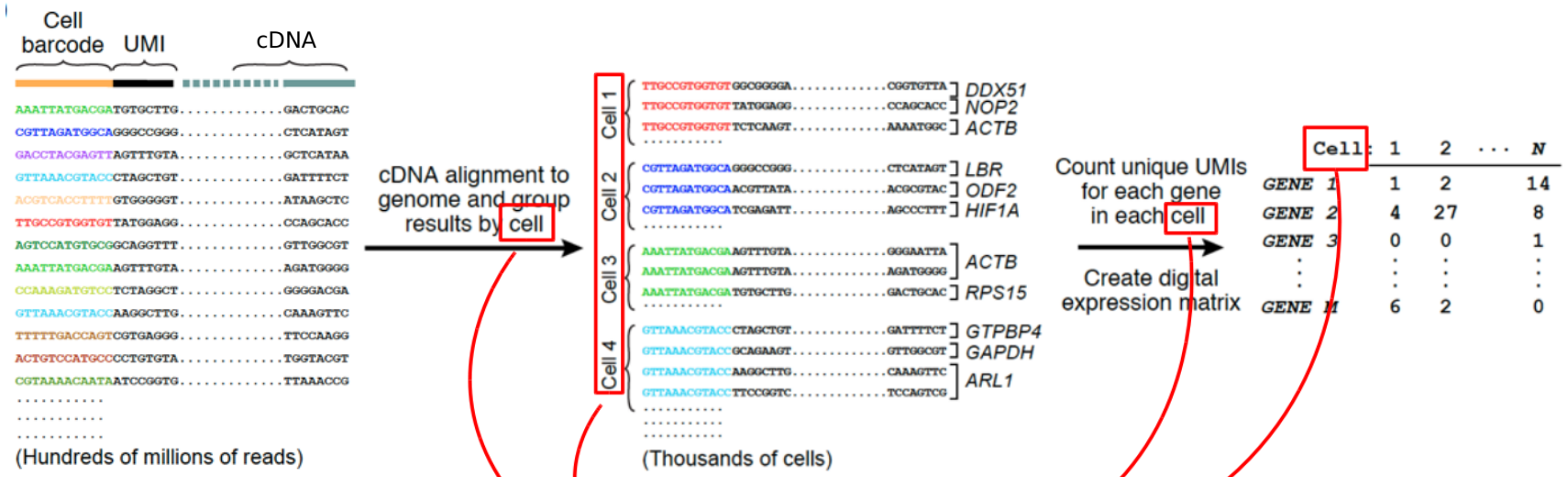
Lack of defined cliff and knee

Metric	Value
Barcodes	> 90,000
Cell Barcodes	~ 10,000
UMIs	> 10,000

Bad!

Problem in cell lysis : RNA not released into the droplet reaction mix. Almost only noise, low signal (Corresponds to the bottom knee on the first.)

Filtering droplets

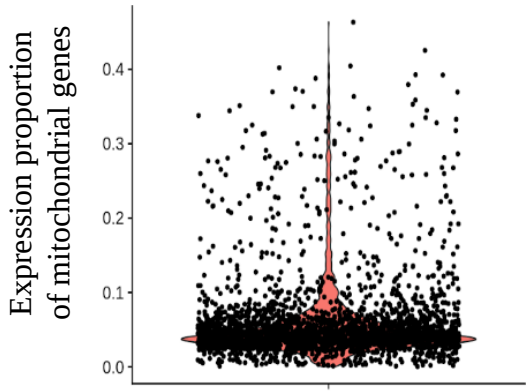


Cells?

Now, sure !

Filtering cells

Mitochondrial genes expression

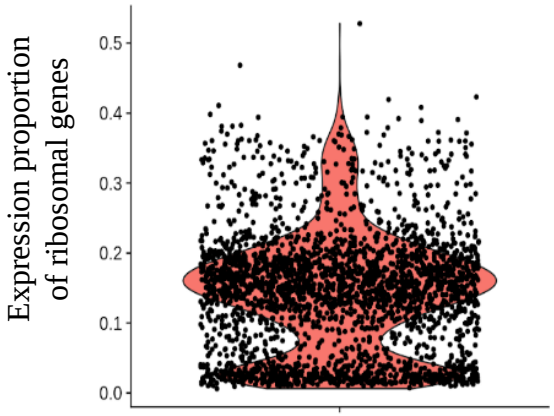


High percentage of mitochondrial genes expression may be due to apoptotic cells :

Kept cells < 5-20% mtRNAs

Gene names beginning with “MT-”.

Ribosomal protein genes expression



Linked to: cellular activity? cell cycle? Not very clear!
Community debate, hard to say if it does matter or not.

Kept cells < 25% rbRNAs ?
10% rbRNAs < Kept cells ?

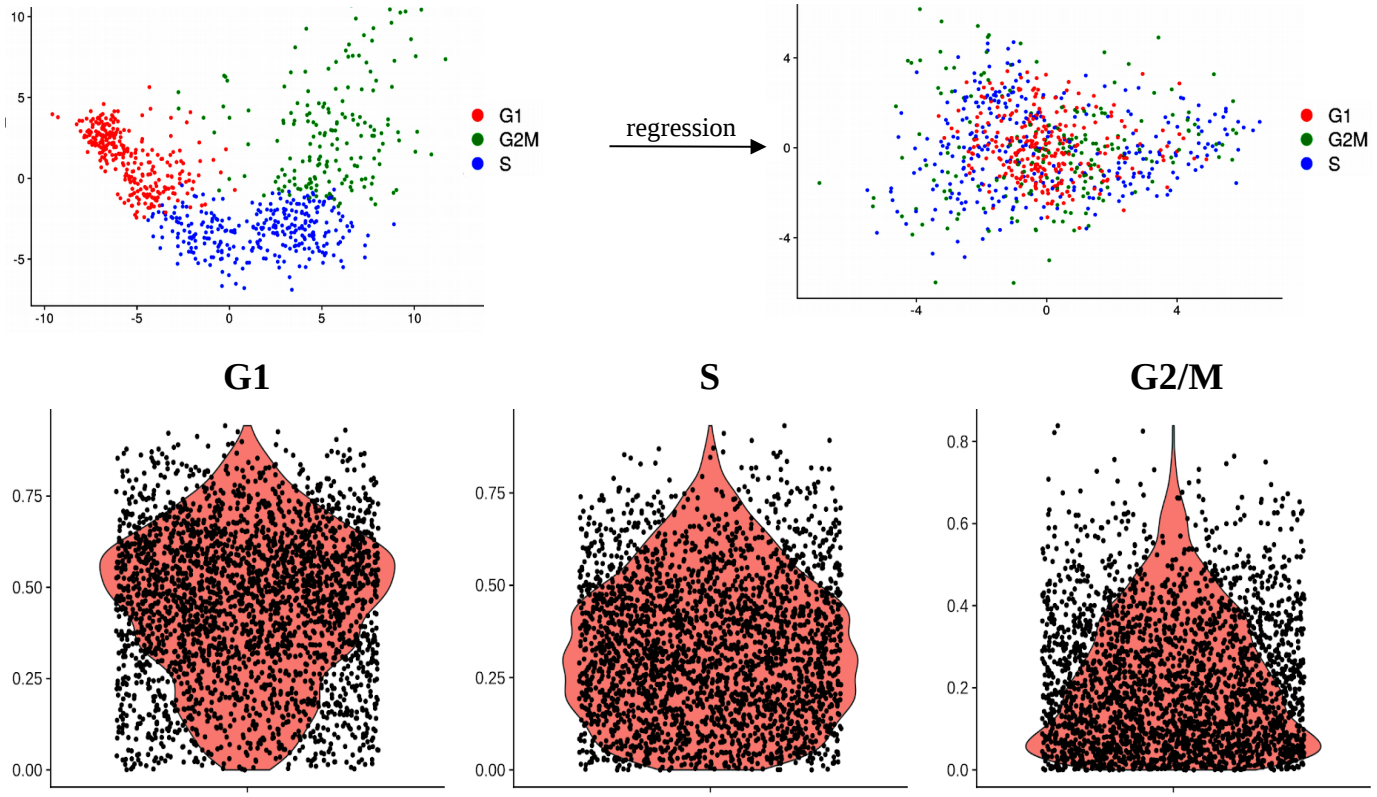
Genes names beginning with “RP-”.

These thresholds are subjective !
Needs to be adjusted according to the biological knowledge of the sample.

R packages: Scater, scRNAseq

Filtering cells

Cell cycle



Cell cycle state of a cell can affect its global gene expression (sometimes strongly), so for a defined cell type, we may observe expression variations linked to the cell cycle that mask other biological signals.

1) Estimation of a cell-cycle score of each cell, then label a cycle stage.
2) Normalization (regression of the score or stage).

R packages: Scran (function cyclone), seurat

Practical Part

Prepare your work environment

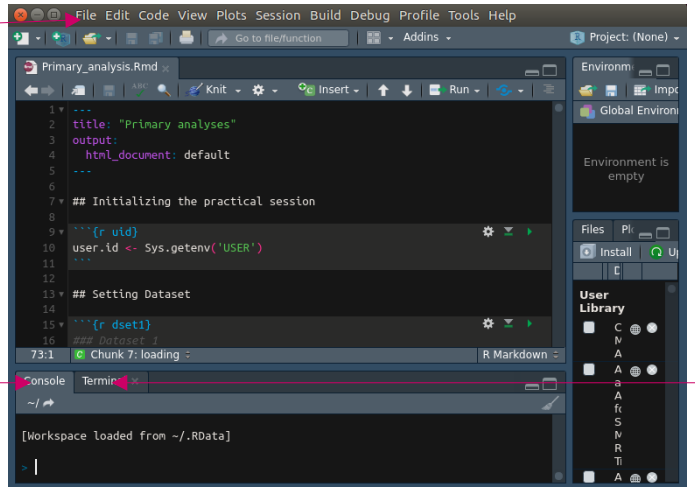
In a bash terminal :
1) Create your working folder
> mkdir -p /shared/projects/sincellte_2022/${USER}/Primary_analyses/

2) Copy scripts
> cp -r /shared/projects/sincellte_2022/Courses/Primary_analyses/scripts /shared/projects/sincellte_2022/${USER}/Primary_analyses/scripts

3) Link datasets
> ln -s /shared/projects/sincellte_2022/Courses/Primary_analyses/input /shared/projects/sincellte_2022/${USER}/Primary_analyses/input

In the RstudioServer console :
1) Go to your working directory
> user.id <- Sys.getenv('USER')
> setwd(paste0("/shared/projects/sincellte_2022/", user.id, "/Primary_analyses"))

2) Open script



Console

Terminal

Script

1) Setting Dataset, Parameters, Seed and Loading R packages
2) Loading data
3) Removing empty droplets :
    a) Automatically: emptydrops()
    b) Manually: raw threshold
4) Computing basic metrics :
    a) Percentage of mito + Percentage of ribo
    b) Identification of background genes
    c) Cell cycle prediction
    d) Identification of doublets
5) Filtering
6) Checking the effect of filtering
7) Save Results

Dataset : description

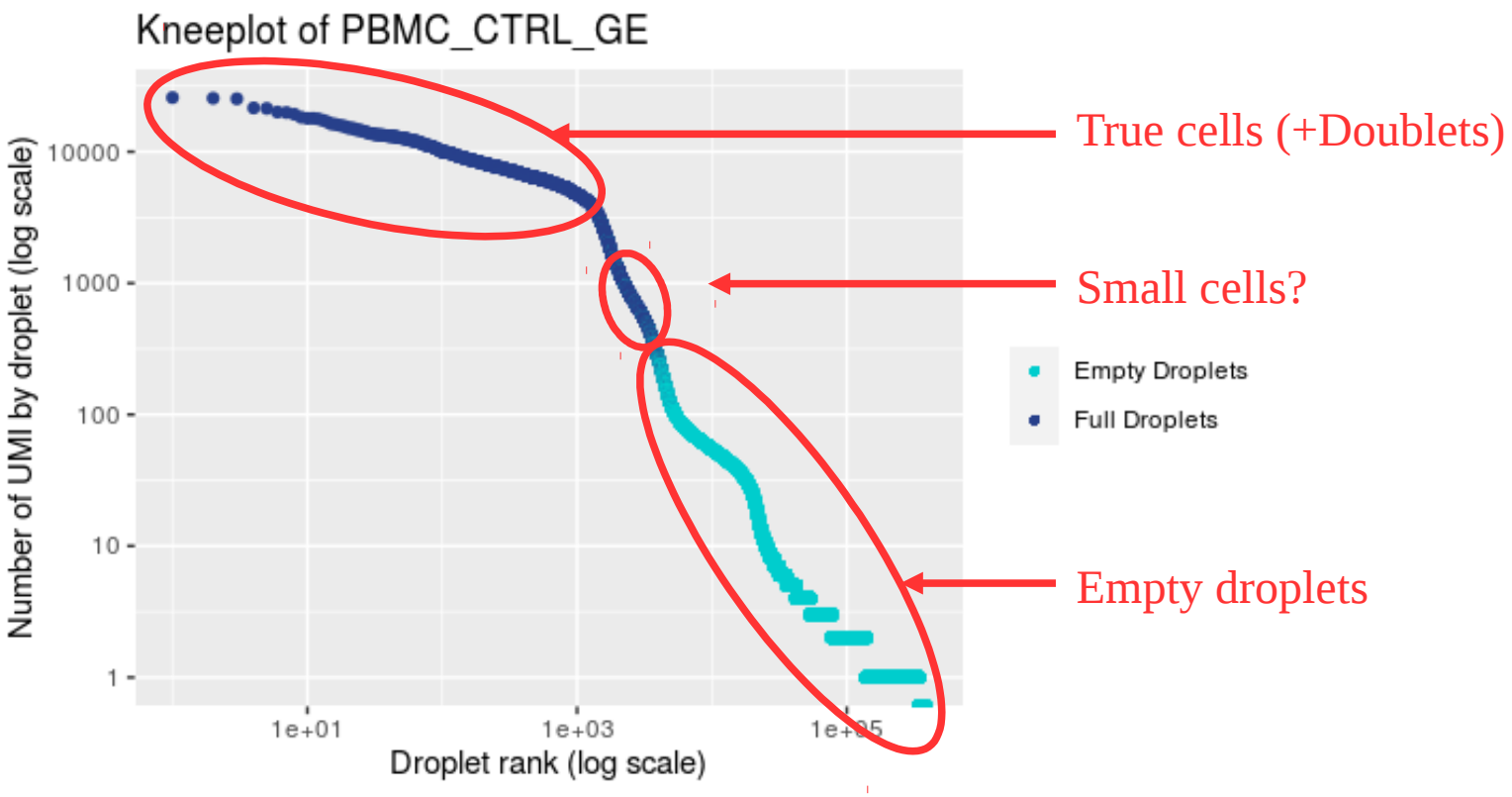
Goal:
Identify the different cell types.

Data information *:*
Organism: Human
Type of tissue collected: Peripheral blood mononuclear cells.
Origin: patient.
Cells treatment: no treatment, healthy cells.
Technology : 10X Genomics Chromium

Expected Result:
We expect about **ten** cell types.

Input type:
Raw, unfiltered counts table from CellRanger.

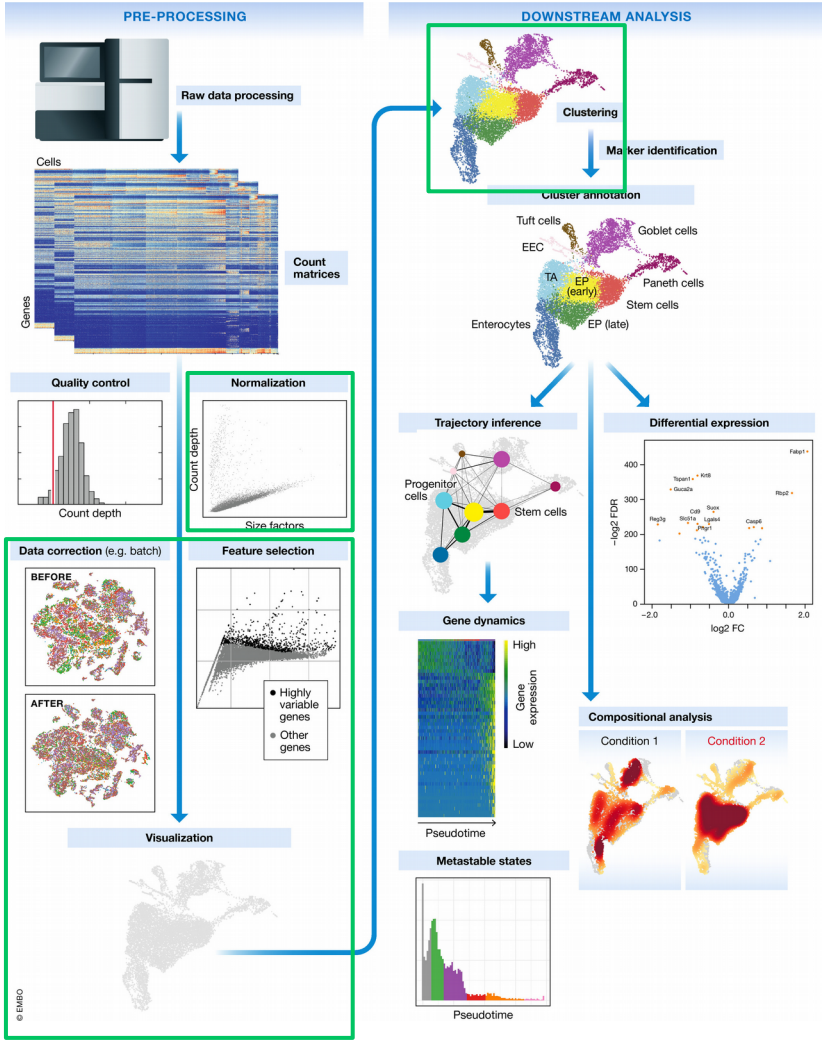
Dataset : results



Here we will make 2 different versions of the results (see html files):
- **with** this population of small cells
- **without** this population of small cells

Main steps of single cell data processing

Tomorrow morning



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