Single-cell RNA-Seq data analysis

Data Processing

FQs

Alignment and counting — *Cell Ranger*

QC Report

Counting matrix

QC assessment

alignment rate → **low** → Check reference genome

Saturation curve → **Not saturate** → Sequence more…

Reads in cells → **low**

knee plot → **atypical**

Correct counts — *SoupX*

Correct cell number and counts — *CellBender*

Corrected matrix

Data processing by Seurat with all default settings — *Seurat*

Detect doublets — *Scrublet*

Seurat object

Load matrix into Seurat

Seurat object

Remove doublets and debris

Marker genes → **Non-specific marker gens**

UMAP and clustering → **Poor clustering**

UMIs, Features, mt content

Re-process with SoupX or CellBender

Optimization

Filtered Seurat object → Normalization → Linear dimensional reduction — *PCA* → Select PCs — *Elbow plot*

Log normalize SCTransform

Merge multiple datasets — *RPCA*

Non-linear dimensional reduction — *UMAP TSNE*

Meta data info

Dimensional reduction info

Optimized Seurat object ← Find optimal resolutions — *clustree*

More analysis

LOUPE file → Import Seurat info into LOUPE file

Customized LOUPE file

Advanced analysis
- *Marker gene identification*
- *Cell type annotation*
- *Differential gene expression*
- *Trajectory analysis*
- …