## scRNA-seq snakemake pipeline manual

Debojyoti Das

25-11-2024

## **Introduction:**

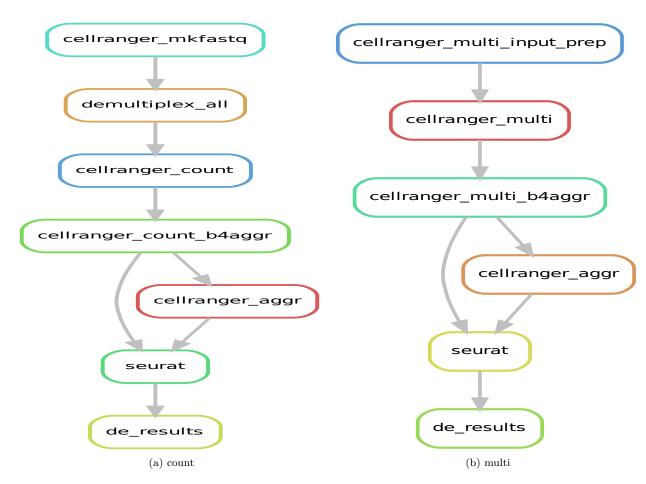


Figure 1: pipeline

The single-cell RNA pipeline can start from either bcl files with initial demultiplexing or already demultiplexed fastq files.

Based on the data format at the start

## **BCL** files

Please prepare a csv file in one of the following formats:

[a] pipeline count: gene expression-only data

```
feature_type,run_bcl_path,iem_samplesheet,samplesheet_4_bcl,fastq_outdirectory,bcl_run_index
"Gene Expression",/path/bcl/folder1,False,/path/to/samplesheet/for/bcl/folder1,/path/to/fastq/output/dir
"Gene Expression",/path/bcl/folder2,True,/path/to/samplesheet/for/bcl/folder2,/path/to/fastq/output/dir
```

[b] pipeline multi: gene expression and VD(J) data

feature\_type,run\_bcl\_path,iem\_samplesheet,samplesheet\_4\_bcl,fastq\_outdirectory,bcl\_run\_index
"Gene Expression,/path/bcl/folder1,False,/path/to/samplesheet/for/bcl/folder1,/path/to/fastq/output/dir
"VDJ-B",/path/bcl/folder2,False,/path/to/samplesheet/for/bcl/folder2,/path/to/fastq/output/vdj\_bcell,rus"
"VDJ-T",/path/bcl/folder3,False,/path/to/samplesheet/for/bcl/folder3,/path/to/fastq/output/vdj\_tcell,rus

/path/to/samplesheet/for/bcl/folder1, the bcl samplesheet can either be a simple samplesheet (iem\_samplesheet False)

```
Lane, Sample, Index
1, test_sample_atac, SI-NA-A1
```

or

an Illumina Experiment Manager file (iem\_samplesheet **True**)

```
[Header],,,,,,,
IEMFileVersion,4,,,,,,
Investigator Name,rjr,,,,,,
Experiment Name, hiseq_test,,,,,,
Date,8/15/16,,,,,,
Workflow, Generate FASTQ,,,,,,
Application, HiSeq FASTQ Only,,,,,,
Assay, TruSeq HT,,,,,,
Description, hiseq sample sheet,,,,,,
Chemistry, Default,,,,,,
[Reads],,,,,,,
26,,,,,,,
98,,,,,,,
,,,,,,,
[Settings],,,,,,,
,,,,,,,,
[Data],,,,,,,
Lane, Sample_ID, Sample_Name, Sample_Plate, Sample_Well, I7_Index_ID, index, Sample_Project, Description
1,s1,test_sample,,,SI-P03-C9,SI-P03-C9,p1,
```

## **FASTQ** files

Please prepare csv file with the following format:

```
fastq,sample,library_type
/path/to/sampl1_fastq_directory/,sample1,Gene Expression
/path/to/sampl2_fastq_directory/,sample2,Gene Expression
/path/to/sampl3_fastq_directory/,sample3,Gene Expression
/path/to/sampl4_fastq_directory/,sample4,Gene Expression
```