

Introduction to scRNA-seq Data Analysis

7/20/2022

Hands on tour of the single cell analysis journey

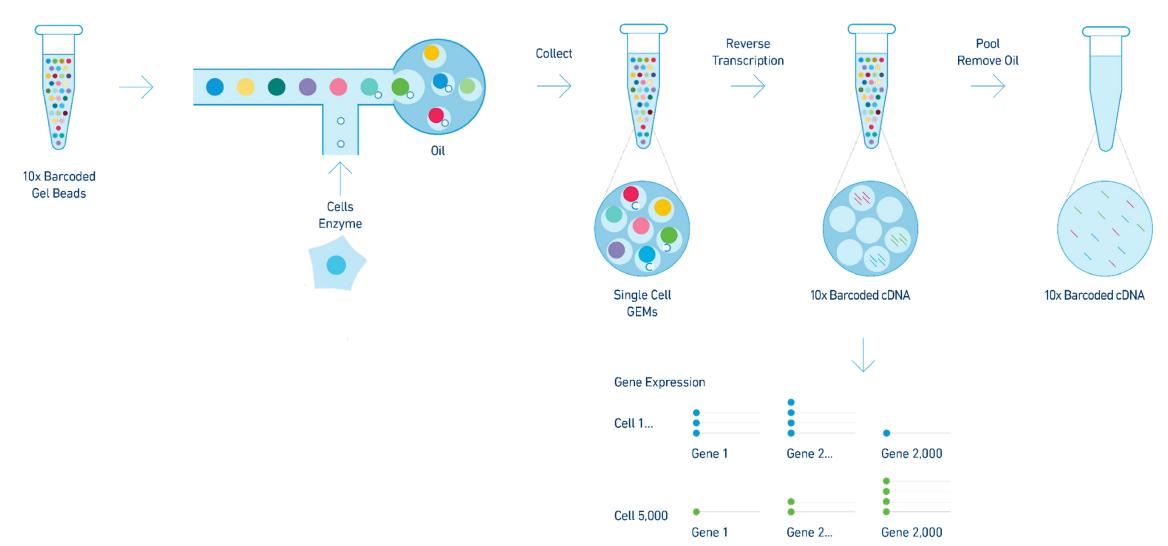
Outline

- Introduction to scRNA-seq
- Overview of Cell Ranger
- Where are we in our neutrophil analysis



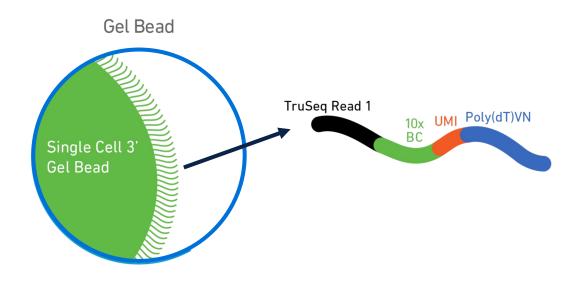
Introduction to scRNA-seq data analysis

10x Genomics 3' Single Cell Gene Expression Assay





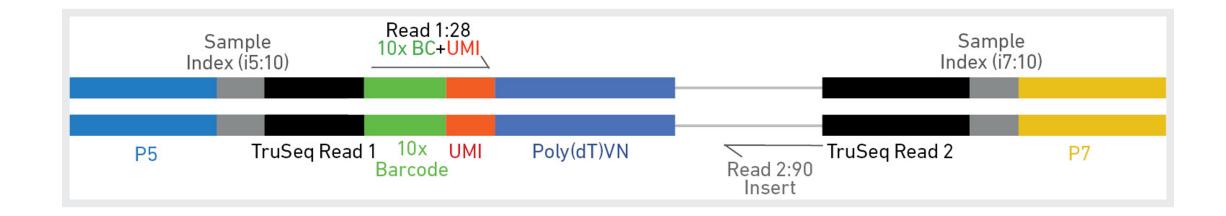
3' Single cell gene expression





Read structure

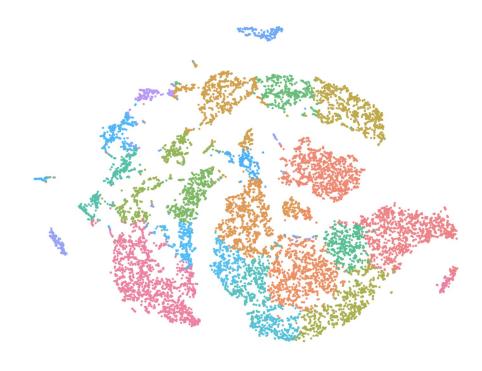
Single Cell 3' Gene Expression library





Common questions answered with scRNA-seq

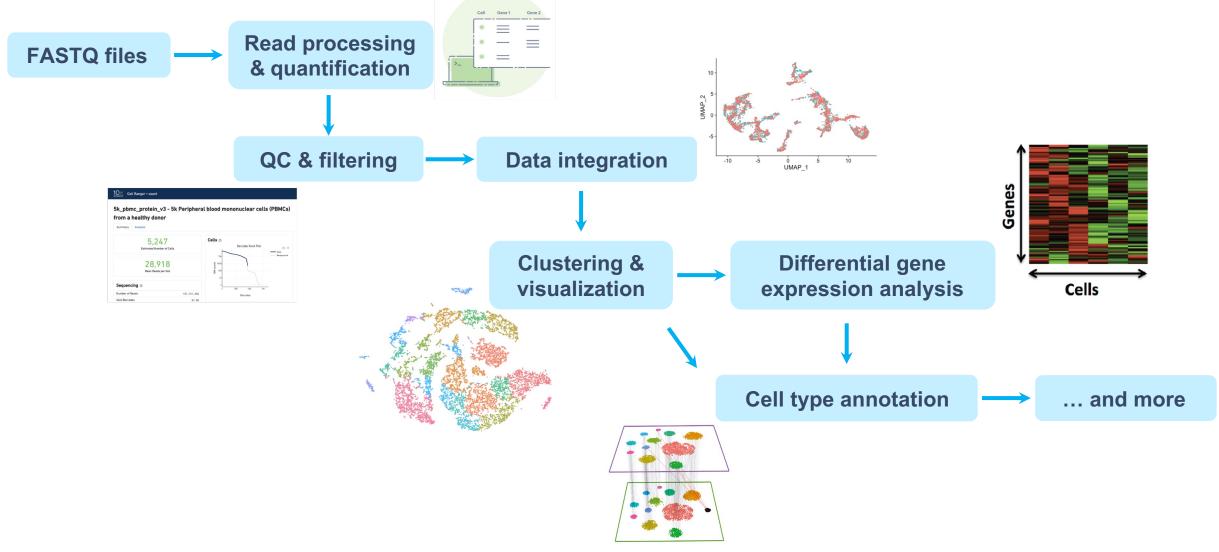
- What gene markers are expressed in different cell populations?
- What cell types are present in my sample? Are there novel or rare cell types?
- What pathways are activated?
- How is the cell population in one sample different from the other (e.g. control vs. drug treatment)?
- How do cells transition from one state to another during development?
- How do cells communicate with each other?



Mouse intestine, ~6600 cells



Single cell gene expression - data analysis flow





Tools in the data analysis flow

Cell Ranger

- A collection of pipelines for processing 10x single cell data
- Developed by 10x Genomics

Loupe

- Desktop tool for analysis and visualization
- Developed by 10x Genomics

Community developed tools

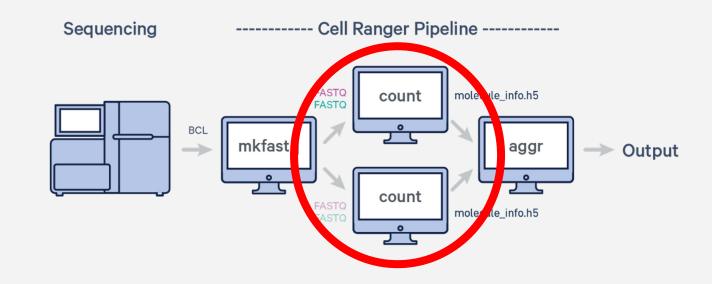
- Primarily programming libraries with some stand-alone tools (e.g. Seurat)
- Developed by the broader research community
- Not officially supported by 10x Genomics



Overview of Cell Ranger

Cell Ranger introduction

- A suite of analysis pipelines that process Chromium Single Cell data
- Contains various pipelines for:
 - Demultiplexing (mkfastq)
 - Single sample analysis (count)
 - Combining data from multiple samples (aggr)
 - Reanalyzing data (reanalyze)





Running Cell Ranger

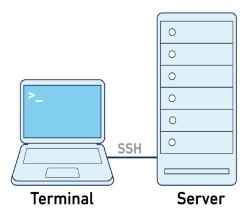
Required skills



On 10x Cloud: Friendly user interface and simple data management (Available only in the US and Canada)

Required skill:

Understand the experimental design



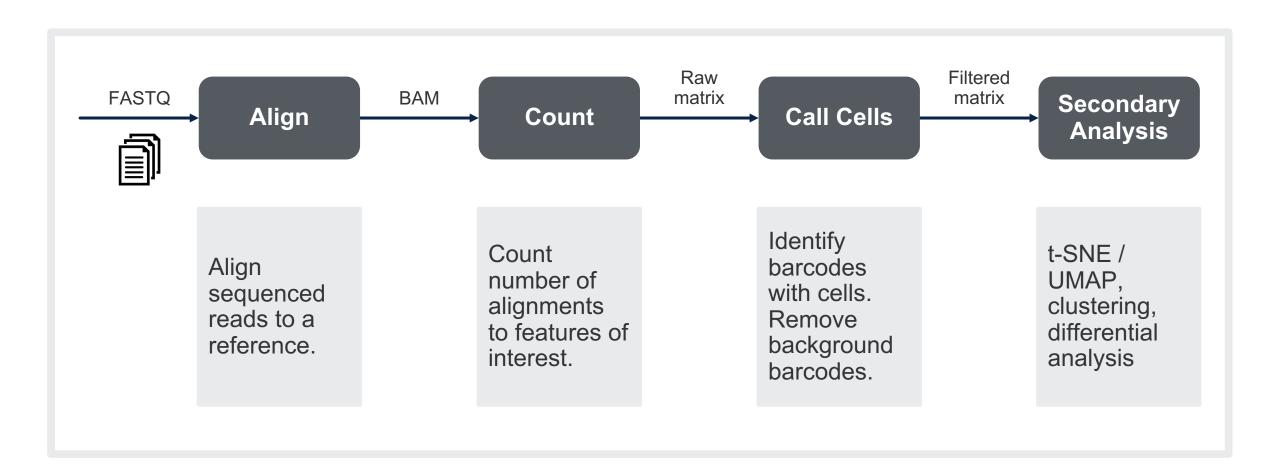
On a Linux system

Required skills:

- Understand the experimental design
- Comfortable running command line in the Linux environment
- Familiar with your organization's data management systems
- Know who to contact if there are server issues



Cell Ranger Analysis Steps





Where are we in our neutrophil analysis

Neutrophil analysis plan

Our journey through analysis

Neutrophils have Neutrophils high Cells separate Separate Neutrophils exist low UMI content intron retention into distinct neutrophils from at different stages of maturation dead/dying cells clusters rates Solution Filter Trajectory Include low Annotate cell Map introns analysis UMI barcodes background clusters **Cell Ranger "force-**Cell Ranger "include-**Loupe Browser Loupe Browser 3rd Party** cells" introns"

