

Welcome to our hands-on tour of the single cell analysis journey

from Cloud to Loupe to community-developed tools

7/20/2022

University of Michigan, Ann Arbor

CG000586 RevA

Welcome! And thank you to our Hosts!

- We are excited to be here and to meet you
- Special thanks to Olivia (Liv) Koues and Chris Gates





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Agenda

Morning: Raw data processing and QA

- Introduction and housekeeping
- 10x Genomics Cloud Analysis
- Introduction to scRNA-seq Data Analysis
- Break
- Quality assessment

Lunch

Afternoon: Downstream analysis and visualization

- Loupe analysis
- Break
- RNA velocity: third party tools
- Planning your individual journey
- Wrap-up



Code of conduct

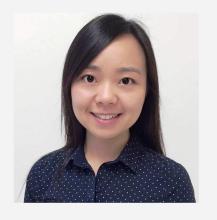
- Ask questions
- Be helpful
- Call out if you get stuck
- Reminder: pre-workshop survey





Workshop team and helpers











Juan

Lisa

Matt







Mike

Nur

Ross



Next up:

10x Genomics Cloud Analysis: from FASTQs to Quantitative Biological Information with Cell Ranger

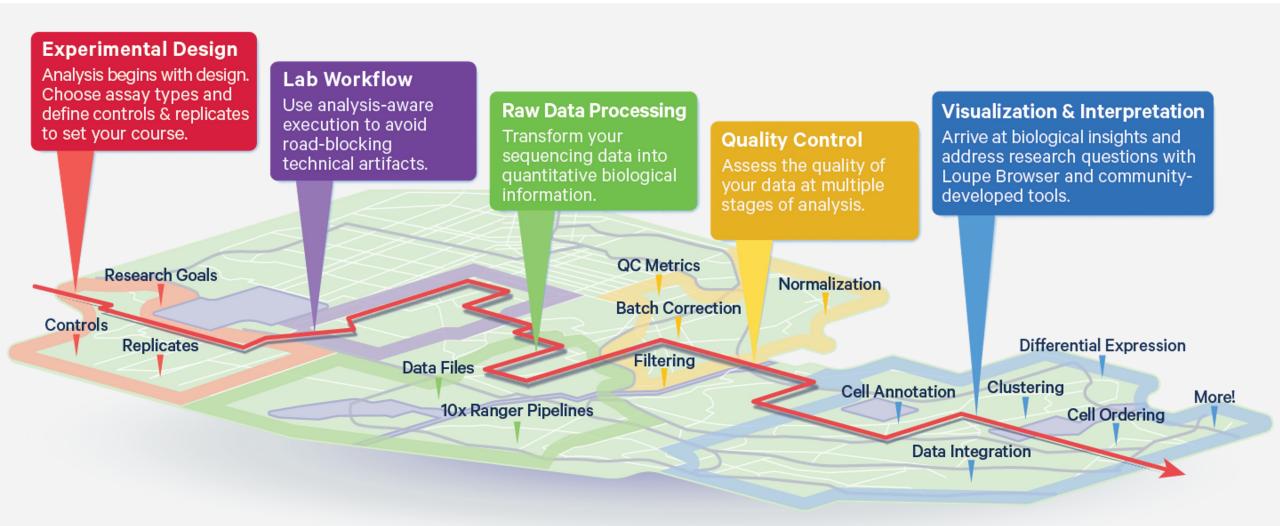
https://web-frontend-6681okbdp-10x-genomics.vercel.app/resources/analysis-guides/workshop-cloud-walkthrough

Topics

- Analysis begins with design: the goals of the experiment will determine how the data should be processed and analyzed.
- Signing in and creating a project
- Using the web based FASTQ uploader
- Creating a new analysis
- Downloading output files



Analysis Journey: Orientation







Introduction to the neutrophil dataset: analysis begins at design

7/20/2022

Hands on tour of the single cell analysis journey

Outline

- Analysis begins at design
- The data we are using today
- Purpose of the experiment
- Neutrophil challenges
- Analysis plan



Analysis begins at design

- The design enables analysis
- What samples, conditions, and data do you need?
- The planning call is the cheapest part of the experiment





The data we are using today

10x Genomics Support / Single Cell Gene Expression / Documentation / Sample Prep /

Neutrophil Analysis in 10x Genomics Single Cell Gene Expression Assays



Neutrophils are the most abundant white blood cells in circulation. They are the first responders of the innate immune system that release cytotoxic compounds to kill bacteria and phagocytose foreign particles. Analysis of neutrophils and granulocytes is challenging in single cell transcriptomic data due to the low RNA content in these cells along with relatively high levels of RNases and other inhibitory compounds. Furthermore, neutrophils are sensitive to degradation after collection, requiring careful sample handling for preservation. This Technical Note describes the successful isolation and analysis of neutrophil populations from whole blood.

Document Type Technical Note

Last Modified

October 25, 2021

 $\underline{https://www.10xgenomics.com/support/single-cell-gene-expression/documentation/steps/sample-prep/neutrophil-analysis-in-10-x-genomics-single-cell-gene-expression-assays}$



Purpose of the experiment

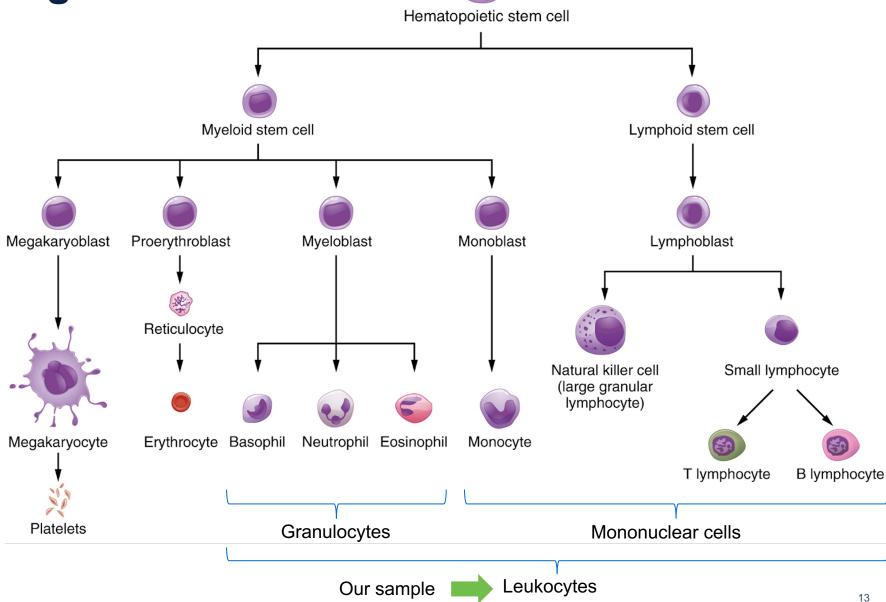
- Show neutrophils are detected by the assay
- Show that the biology is preserved





Neutrophil challenges

- Neutrophils are hard to collect on their own
- Neutrophils have **lower RNA** content and express fewer genes
- Neutrophils have elevated levels of intron retention





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 clusters dead/dying cells of maturation rates Solution Filter Trajectory Include low Annotate cell Map introns UMI barcodes clusters background analysis **Cell Ranger "force-**Cell Ranger "include-**Loupe Browser Loupe Browser 3rd Party** introns" cells"

