10X GENOMICS

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

From Cloud to Loupe to community-developed tools

CG000586 RevB

Goals and objectives

Primary goal

- **Demystify the data analysis process with a hands-on, active learning approach.**

Target audience
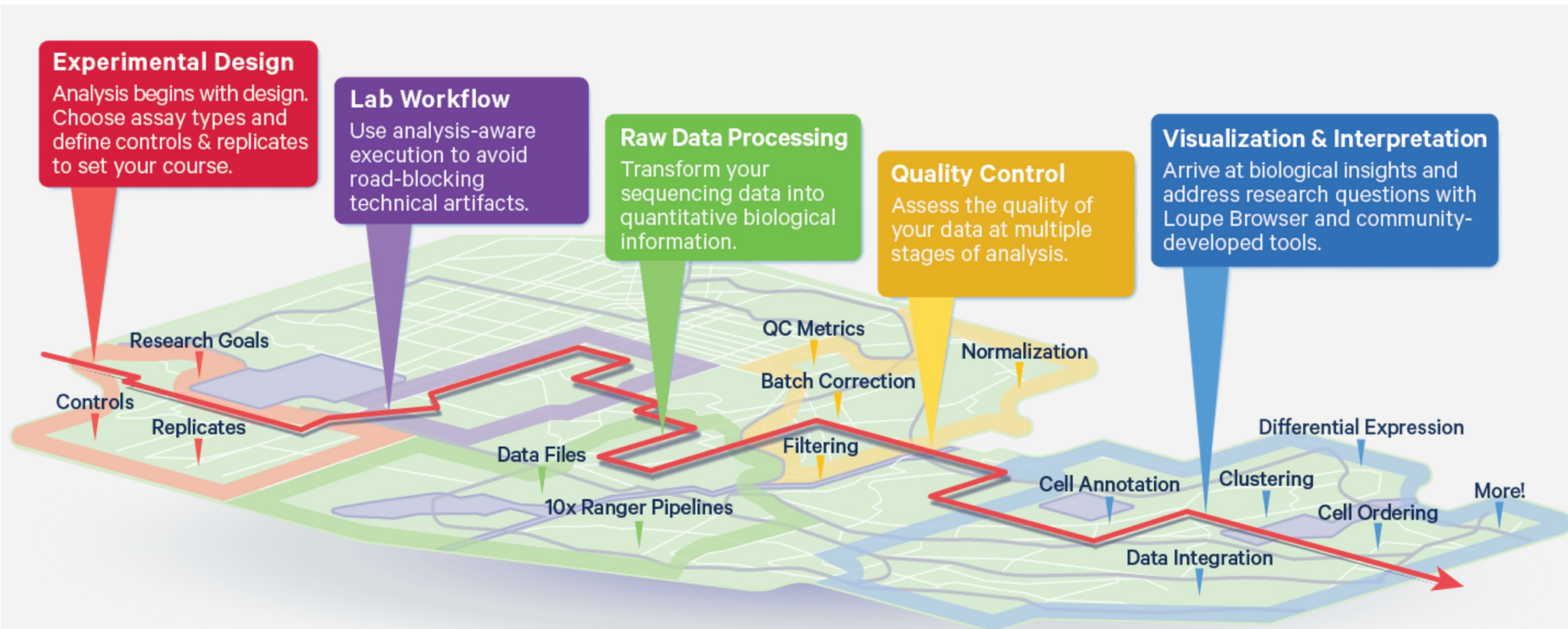
- Those who have or will soon have 10X data and have limited to no bioinformatics experience

Secondary goals

TL;DR: Help you move forward in analysis

- Help you see a path for you through the analysis journey
- Bring awareness to useful analysis resources, such as software documentation and analysis guides
- Get you started in raw data processing using 10x cloud analysis
- Show the expanded functionality of our Loupe Browser
- Introduce the amazing ecosystem of community developed tools for analyzing 10X data

Analysis Journey: Orientation

Experimental Design
Analysis begins with design. Choose assay types and define controls & replicates to set your course.

Lab Workflow
Use analysis-aware execution to avoid road-blocking technical artifacts.

Raw Data Processing
Transform your sequencing data into quantitative biological information.

Quality Control
Assess the quality of your data at multiple stages of analysis.

Visualization & Interpretation
Arrive at biological insights and address research questions with Loupe Browser and community-developed tools.

Research Goals

Controls

Replicates

QC Metrics

Batch Correction

Normalization

Data Files

Filtering

10x Ranger Pipelines

Cell Annotation

Clustering

Differential Expression

Cell Ordering

More!

Data Integration

Agenda

Raw data processing and QA

- Introduction

- 10x Genomics Cloud Analysis

- Overview of scRNA-seq Data Analysis

- Quality Assessment

Downstream analysis and visualization

- Loupe Analysis

- RNA Velocity: Community Developed Tools

- Planning your Individual Journey

Introduction to the neutrophil dataset: analysis begins at design

Hands on tour of the single cell analysis journey

Outline

- Analysis begins at design

- The data we are using in this series

- 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

https://imgflip.com/memegenerator/One-Does-Not-Simply

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

Technical Note, CG000444

CG000444_Neutrophil Analysis_TN_RevA .pdf

View and download

Document Type

Technical Note

Last Modified

October 25, 2021

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.

https://www.10xgenomics.com/support/single-cell-gene-expression/documentation/steps/sample-prep/neutrophil-analysis-in-10-x-genomics-single-cell-gene-expression-assays
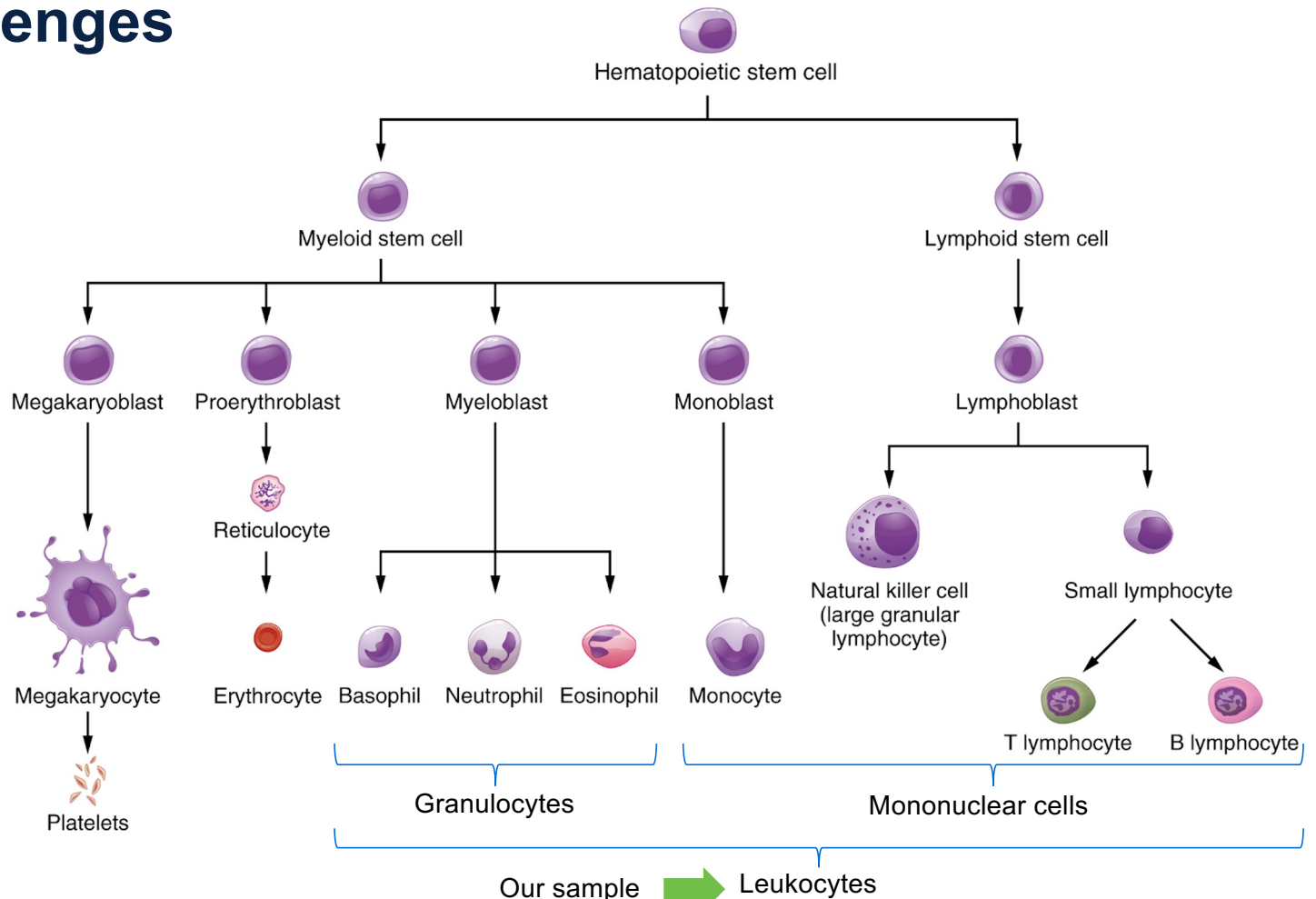
Purpose of the experiment

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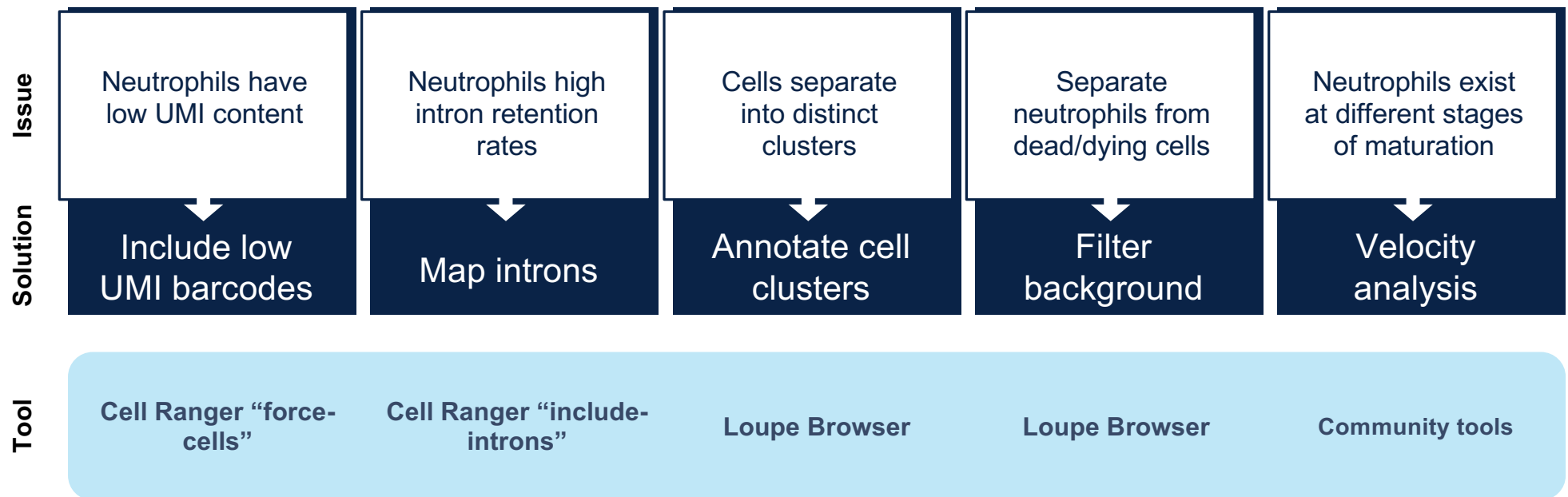
- Show that the biology is preserved

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

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4551565/

Neutrophil analysis plan

Our journey through analysis

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

Next up:

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

Topics

- Analysis begins with design: the goals of the experiment will determine how the data should be processed and analyzed.
- Introduction to the neutrophil data set

Next

- Signing in and creating a project
- Using the web based FASTQ uploader
- Creating a new analysis
- Downloading output files