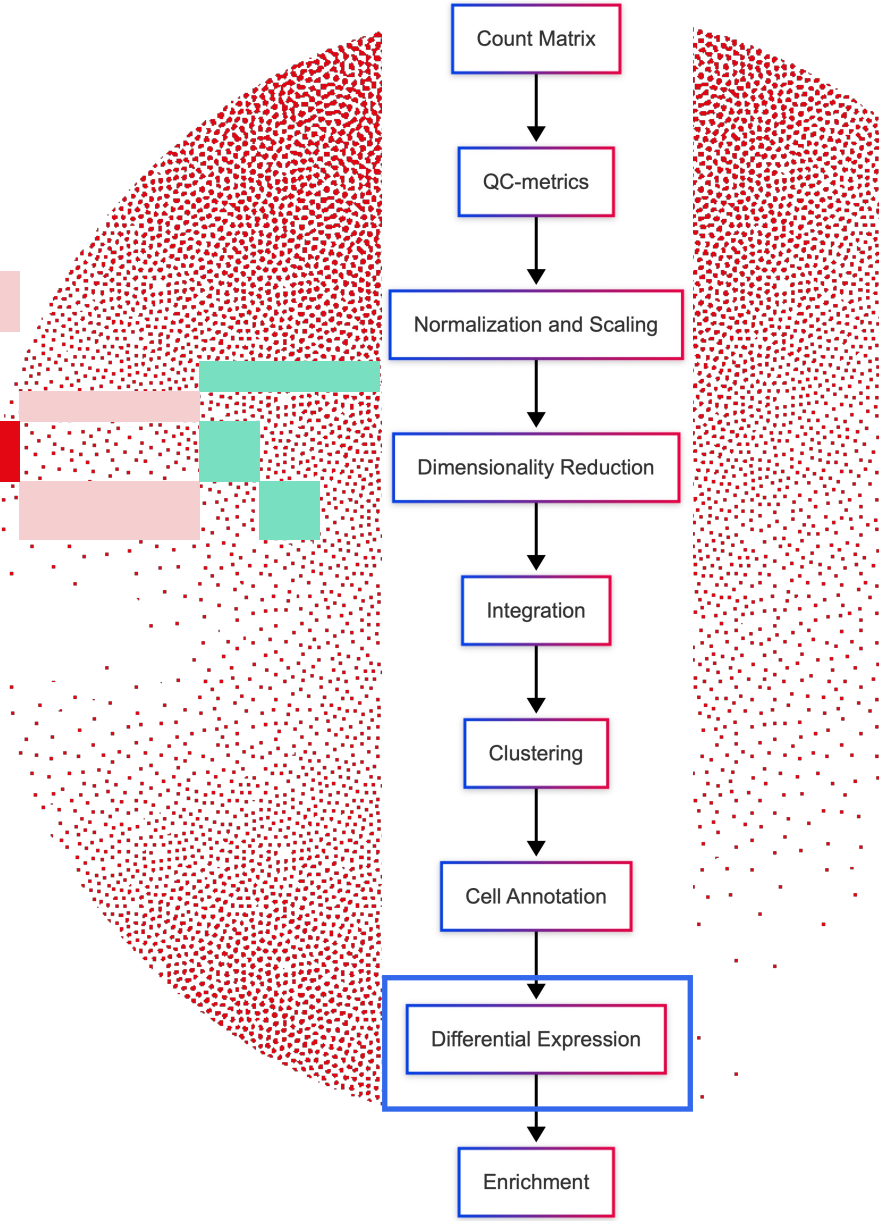
Swiss Institute of Bioinformatics

SINGLE-CELL TRANSCRIPTOMICS WITH R

Differential gene expression analysis



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March 18-20, 2025

Adapted from previous year courses

Feedback from Geert van Geest

elixir SWITZERLAND

Two types of gene expression analysis

1. **Marker gene identification**

Identify genes that are highly expressed in one group of cells (e.g., a specific cell type or cluster) compared to others, often used to annotate cell types or states.

2. **Differential gene expression analysis**

Identify genes that show a different expression between two populations, for example cells or individuals

Marker gene identification

Methods:

- Log-Fold Change (LFC) Analysis: Calculate the log-fold change in expression between a target cluster and all other cells (e.g., in Seurat or Scanpy).

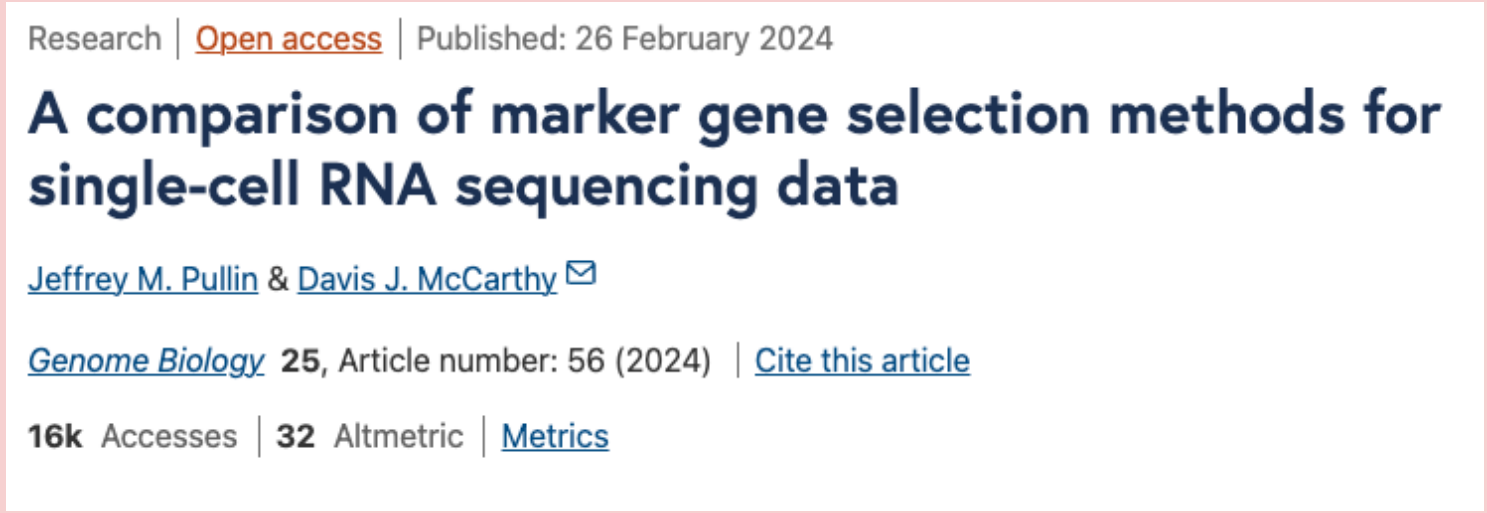
- Wilcoxon Rank-Sum Test: Test for genes with significantly higher expression in one group compared to others (e.g., in Seurat).

Use Cases:

- Annotating cell types (e.g., identifying CD_3 as a marker for T cells)
- Discovering novel cell states or subpopulations

Challenges:

- Marker genes may not be unique to a single cell type, requiring careful validation
- Dropout events can obscure marker gene detection



Research | Open access | Published: 26 February 2024

A comparison of marker gene selection methods for single-cell RNA sequencing data

Jeffrey M. Pullin & Davis J. McCarthy

Genome Biology **25**, Article number: 56 (2024) | Cite this article

16k Accesses | **32** Altmetric | Metrics

Methods based on logistic regression, Student's *t*-test and the Wilcoxon rank-sum test all have strong performance

Differential gene expression analysis

DGE can be divided into 2 sub-groups:

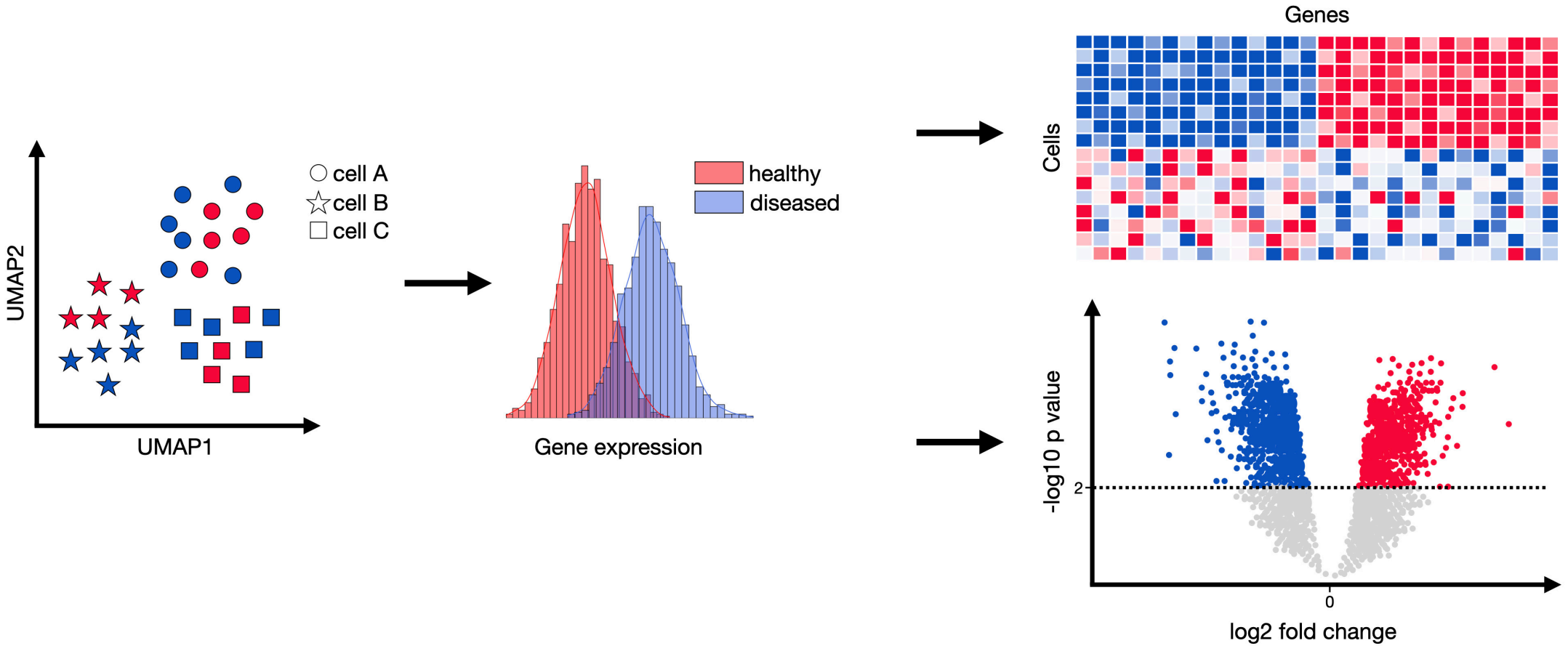
a. Single-Cell-Level DGE Analysis (finding markers)

Goal: Identify genes that are differentially expressed between cell populations (e.g., cell types, clusters, or conditions) while accounting for the single-cell nature of the data (e.g., sparsity, dropout events).

b. Pseudo-Bulk DGE Analysis (differential analysis)

Goal: Aggregate single-cell data into pseudo-bulk profiles to perform DGE analysis using bulk RNA-seq methods, reducing noise and leveraging biological replicates.

a. Single-Cell-Level DGE Analysis



a. Single-Cell-Level DGE Analysis

Methods:

- Wilcoxon Rank-Sum Test: A non-parametric test commonly used to compare gene expression distributions between two groups of cells (e.g., implemented in Seurat via the FindMarkers function).

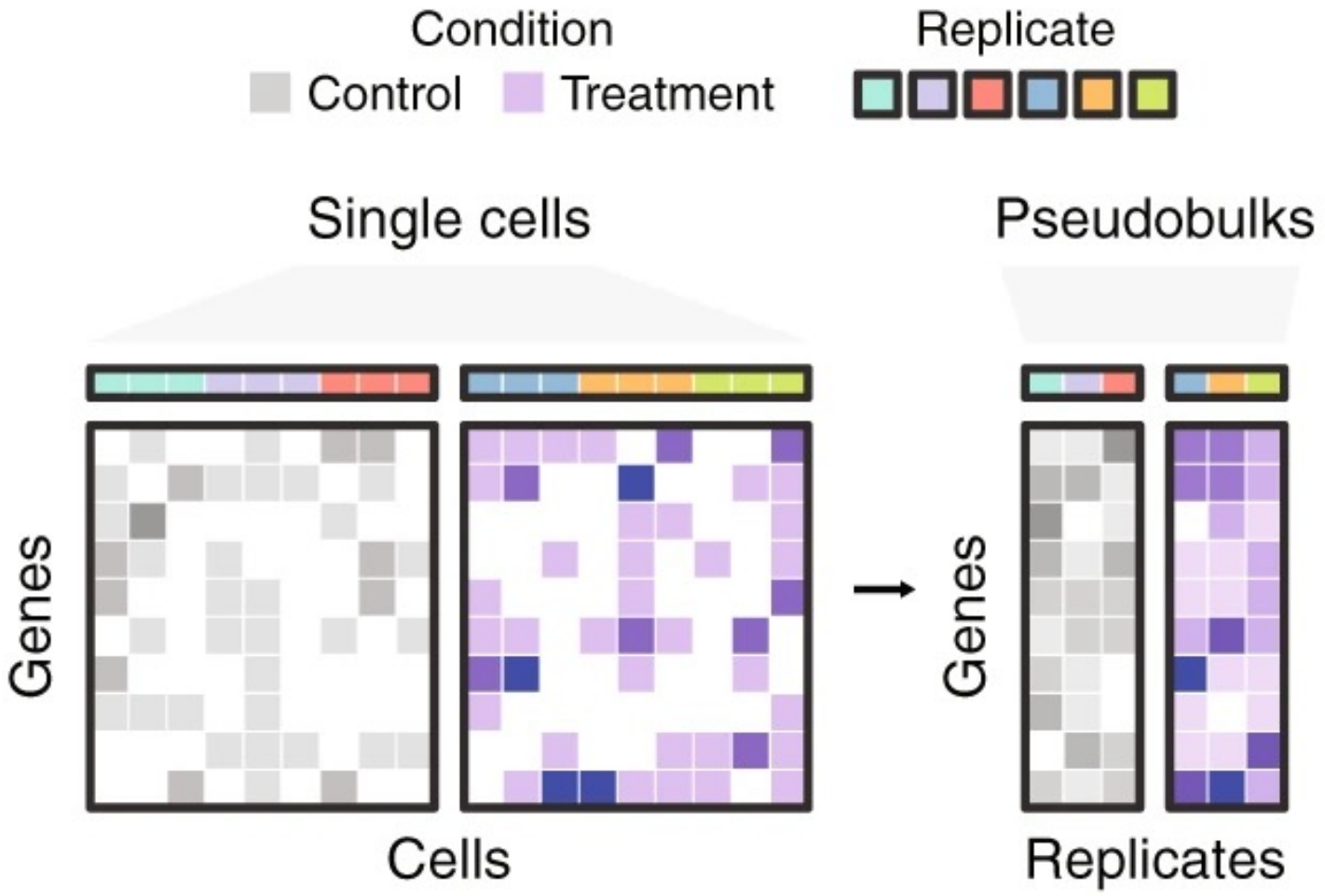
- **MAST (Model-based Analysis of Single-cell Transcriptomics)**: A hurdle model that accounts for dropout events and technical variability in scRNA-seq data.

Challenges:

- High false-positive rates due to the large number of cells and genes tested.

- Dropout events can obscure true biological differences.

b. Pseudo-Bulk DGE Analysis



b. Pseudo-Bulk DGE Analysis

Methods:

- Aggregate gene expression counts within groups (e.g., by cell type and sample) and use bulk RNA-seq tools like DESeq2, edgeR, or limma

- Tools like muscat in Bioconductor are specifically designed for pseudo-bulk DGE analysis in scRNA-seq

Advantages:

- Reduces noise and dropout effects

- Leverages well-validated bulk RNA-seq tools

Limitations: Loses single-cell resolution and cannot detect cell-to-cell variability

Quiz

What is a key limitation of pseudo-bulk analysis compared to single cell-level analysis?

A) It is computationally more intensive.

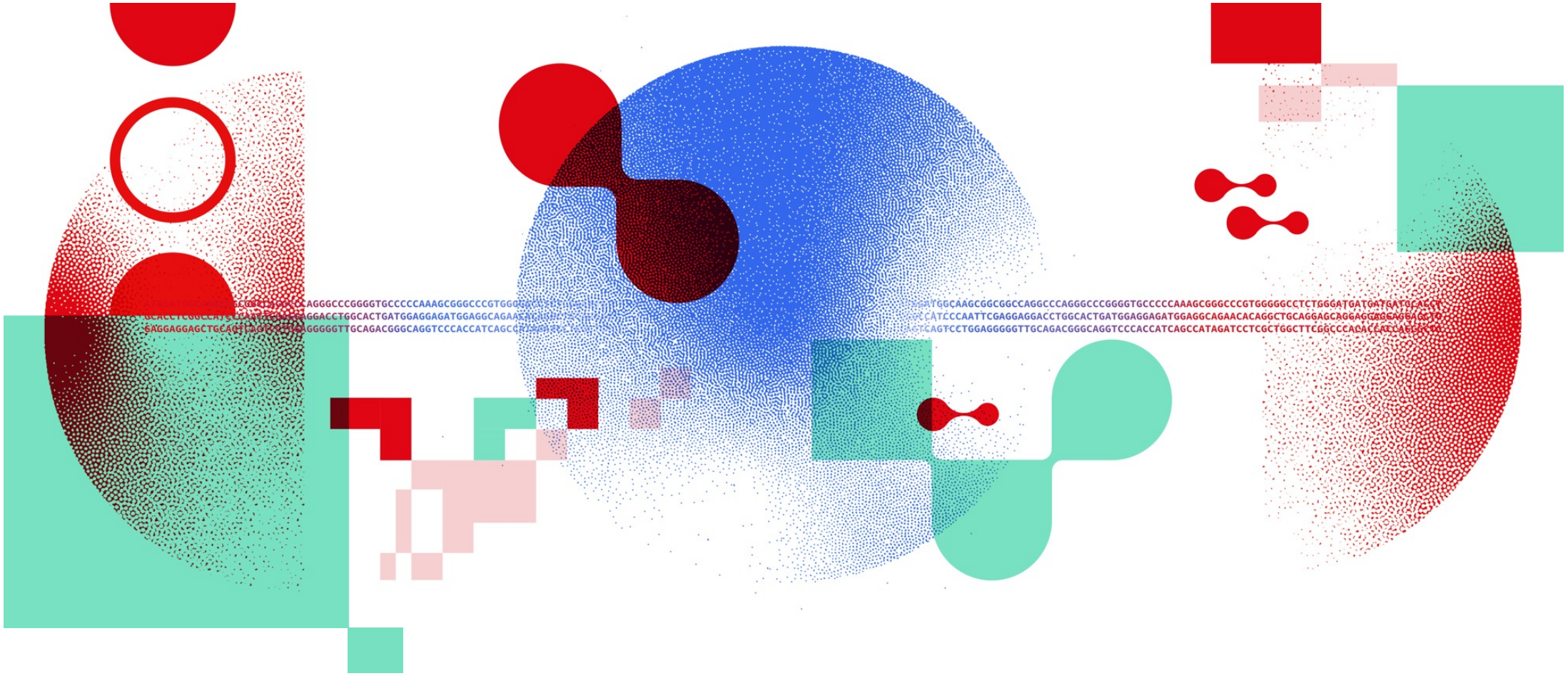
B) It loses single-cell resolution and cannot detect cell-to-cell variability.

C) It cannot be used for differential expression analysis.

D) It is less robust to dropout events.

Single Cell-Level: Finding markers

Pseudo-Bulk: Differential expression



Thank you

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