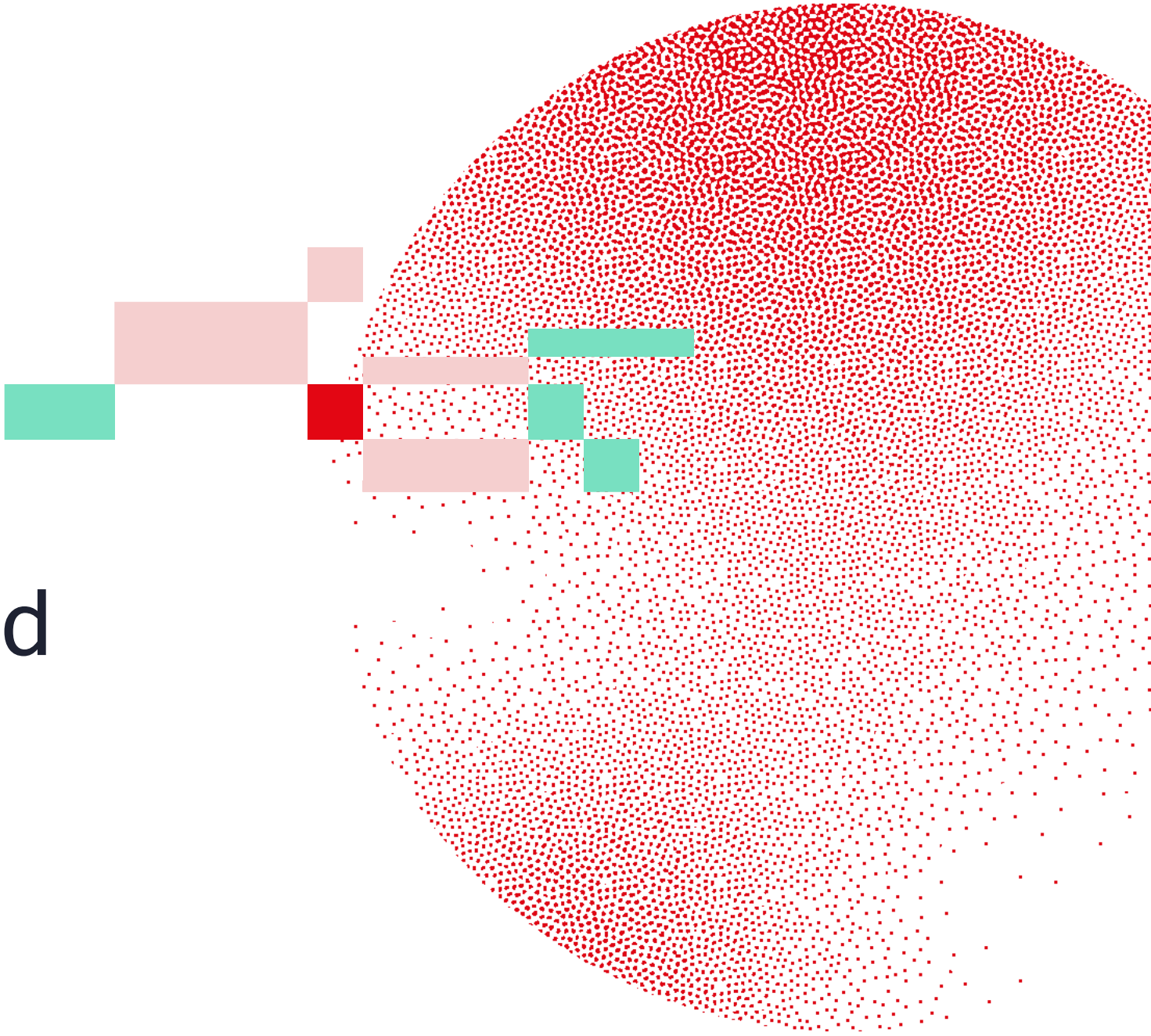
Swiss Institute of Bioinformatics



SINGLE-CELL TRANSCRIPTOMICS WITH R

Normalization, Transformation, and scaling

Deepak Tanwar

March 18-20, 2026

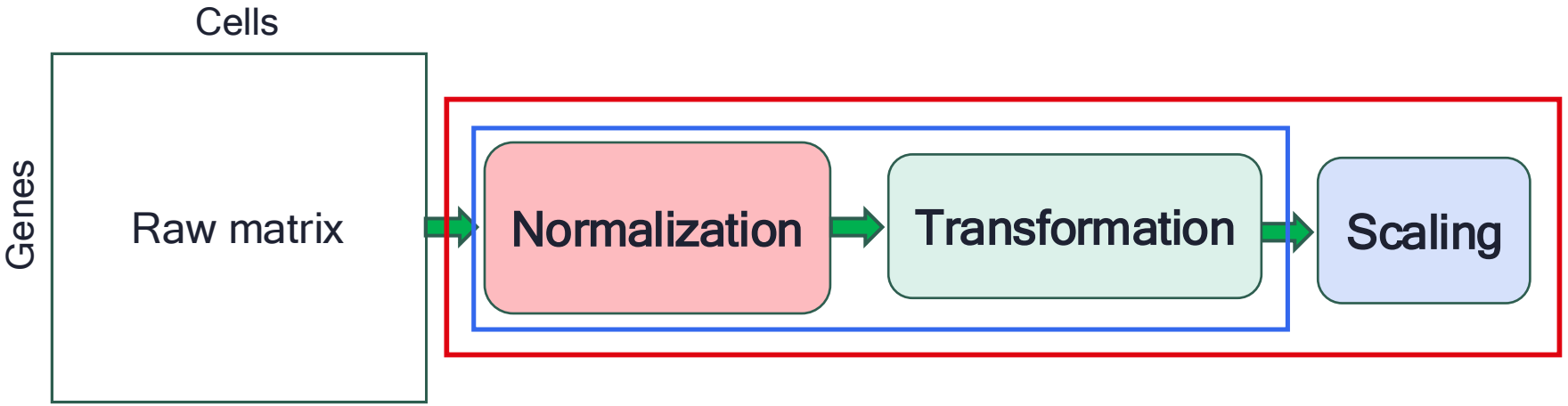
Adapted from previous year courses

Learning objectives

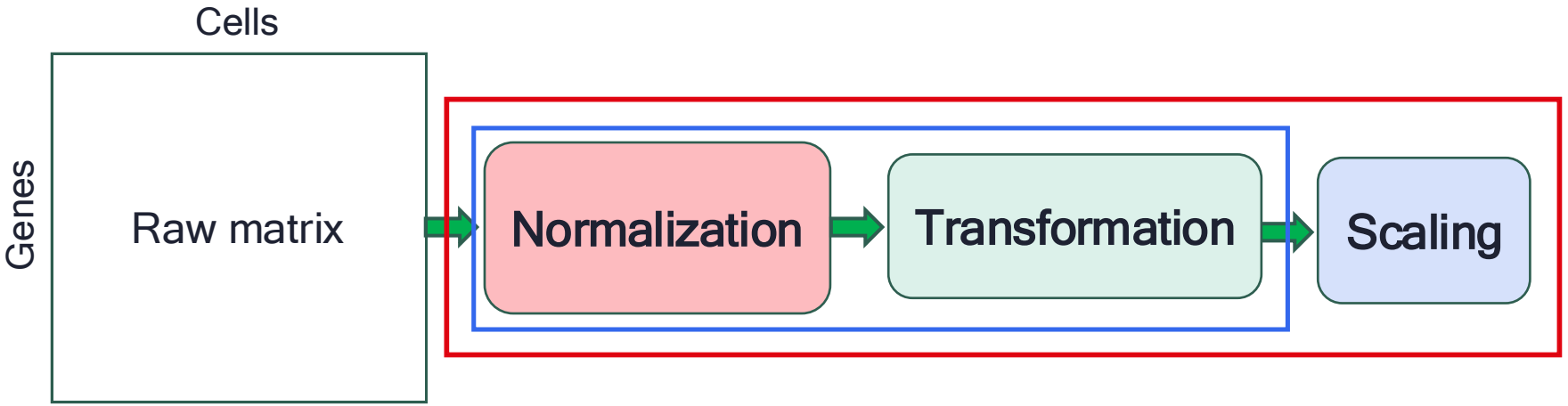
Distinguish Normalization, Transformation, and Scaling

Identify and apply Normalization techniques

Normalization and scaling

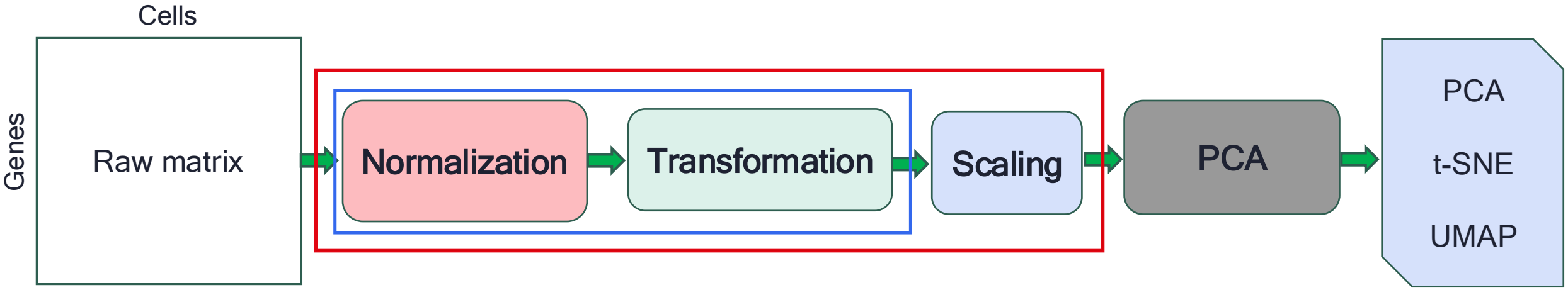


Normalization and scaling

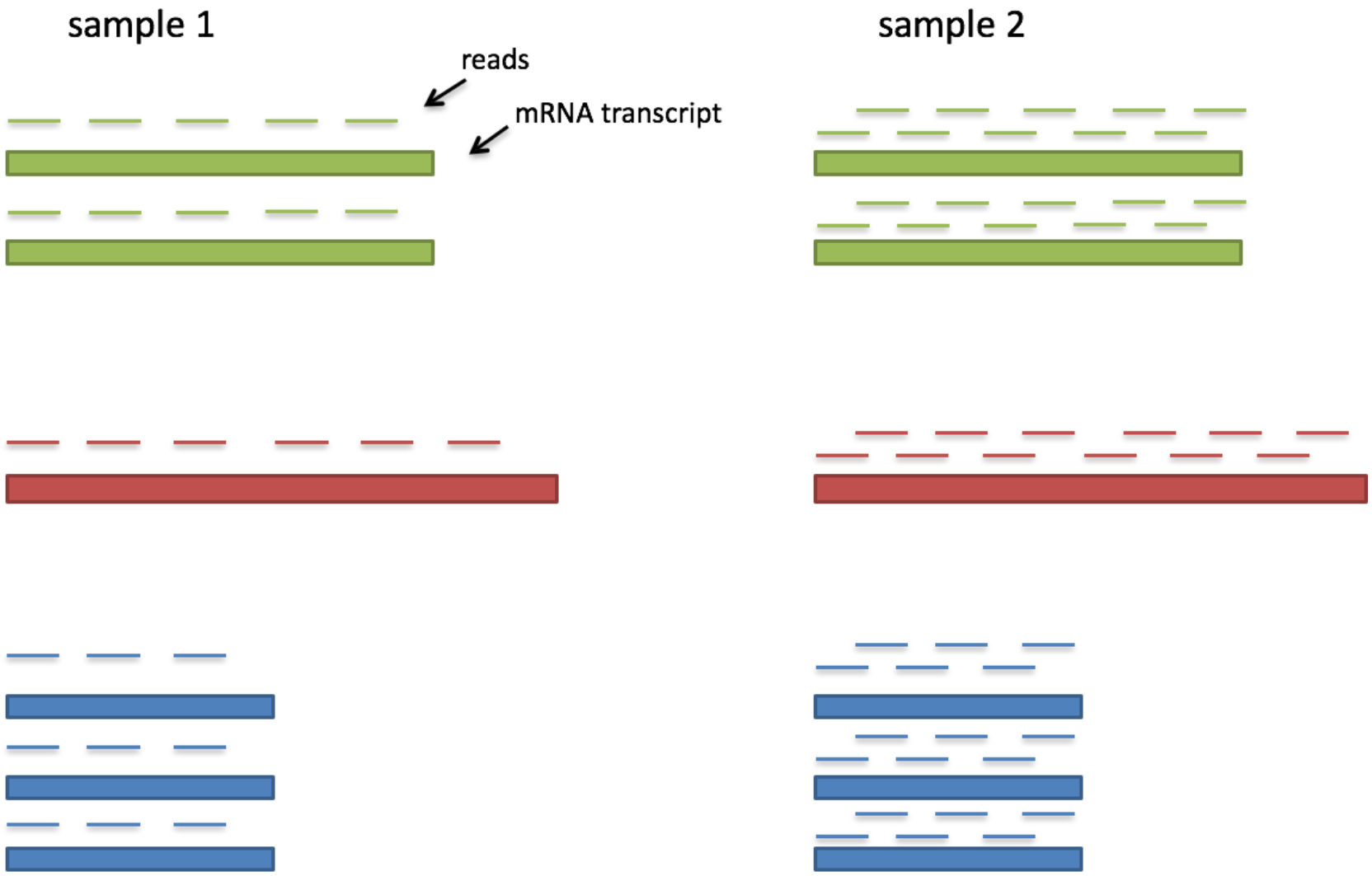


Why am I doing this?

Normalization and scaling



Differences from sequencing data



Problems with raw counts

Problems with raw counts

1. Library size bias → deeper sequenced cells dominate

Problems with raw counts

1. Library size bias → deeper sequenced cells dominate

2. Zero-inflation → most genes = 0

Problems with raw counts

1. Library size bias → deeper sequenced cells dominate
2. Zero-inflation → most genes = 0
3. High dynamic range

Goal of normalization and transformation

1. Remove sequencing-depth (library-size) differences so that cells are comparable.

Goal of normalization and transformation

1. Remove sequencing-depth (library-size) differences so that cells are comparable.

2. Transform values with `log`

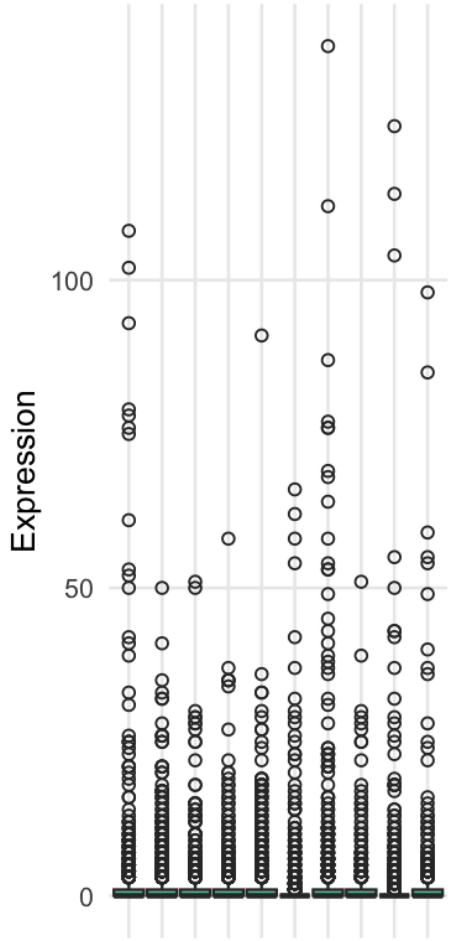
Goal of normalization and transformation

1. Remove sequencing-depth (library-size) differences so that cells are comparable.

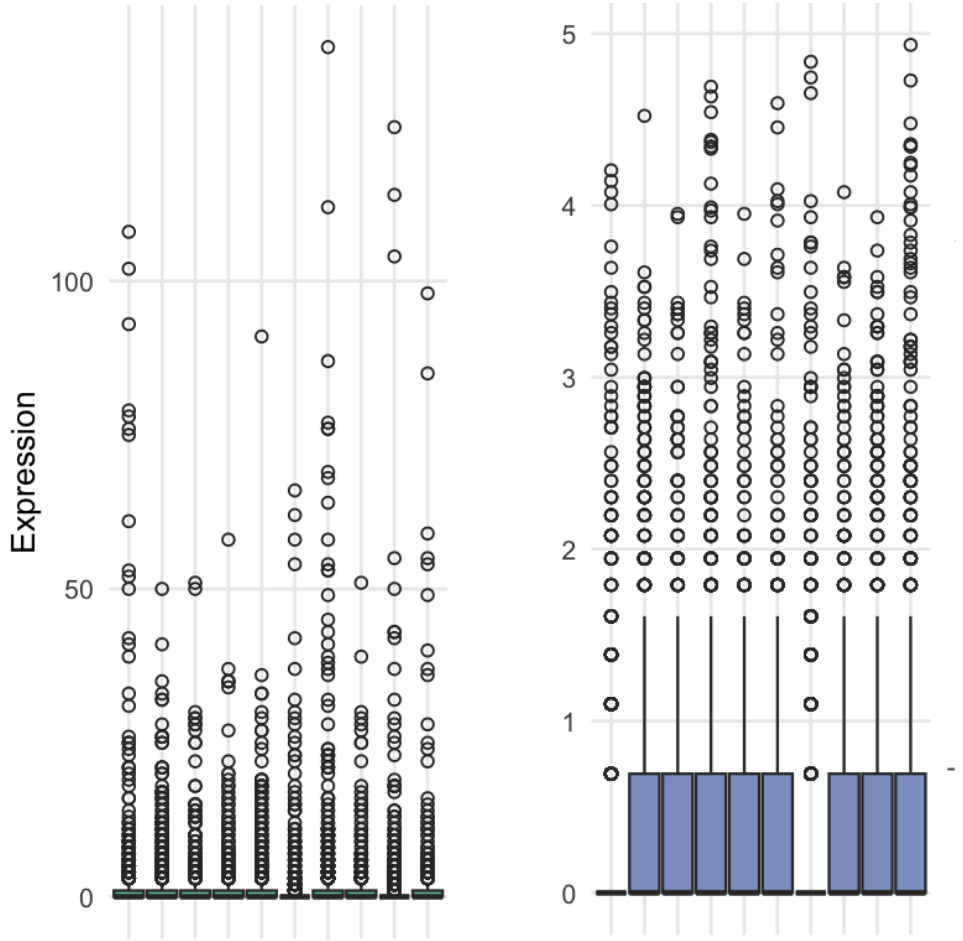
2. Transform values with `log`

3. Multiply each UMI count by 10,000 to make it in a readable/manageable scale

Transformation

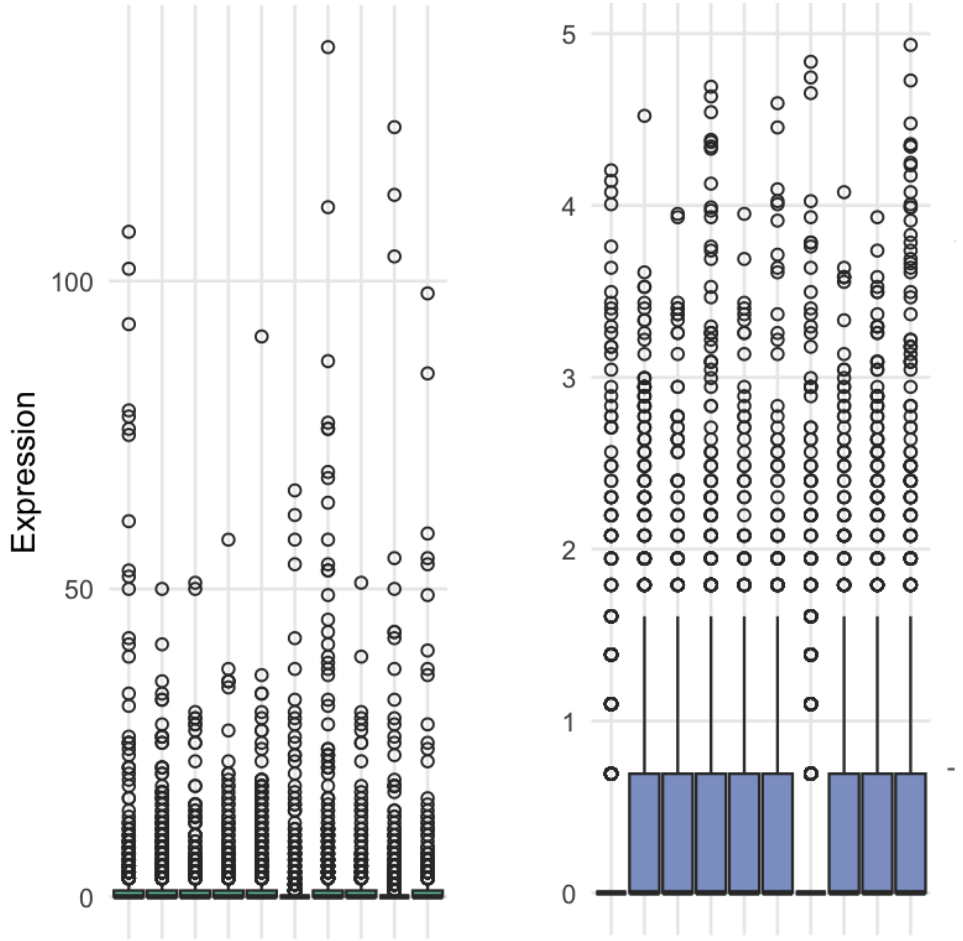


Transformation



$$\text{norm}_{g,c} = \log\left(\frac{\text{count}_{g,c}}{\sum_g \text{count}_{g,c}} \times 10{,}000 + 1\right)$$

Transformation



`Seurat::NormalizeData()`

$$\text{norm}_{g,c} = \log\left(\frac{\text{count}_{g,c}}{\sum_g \text{count}_{g,c}} \times 10{,}000 + 1\right)$$

What Is Scaling?

The Problem: Genes Have Different Scales

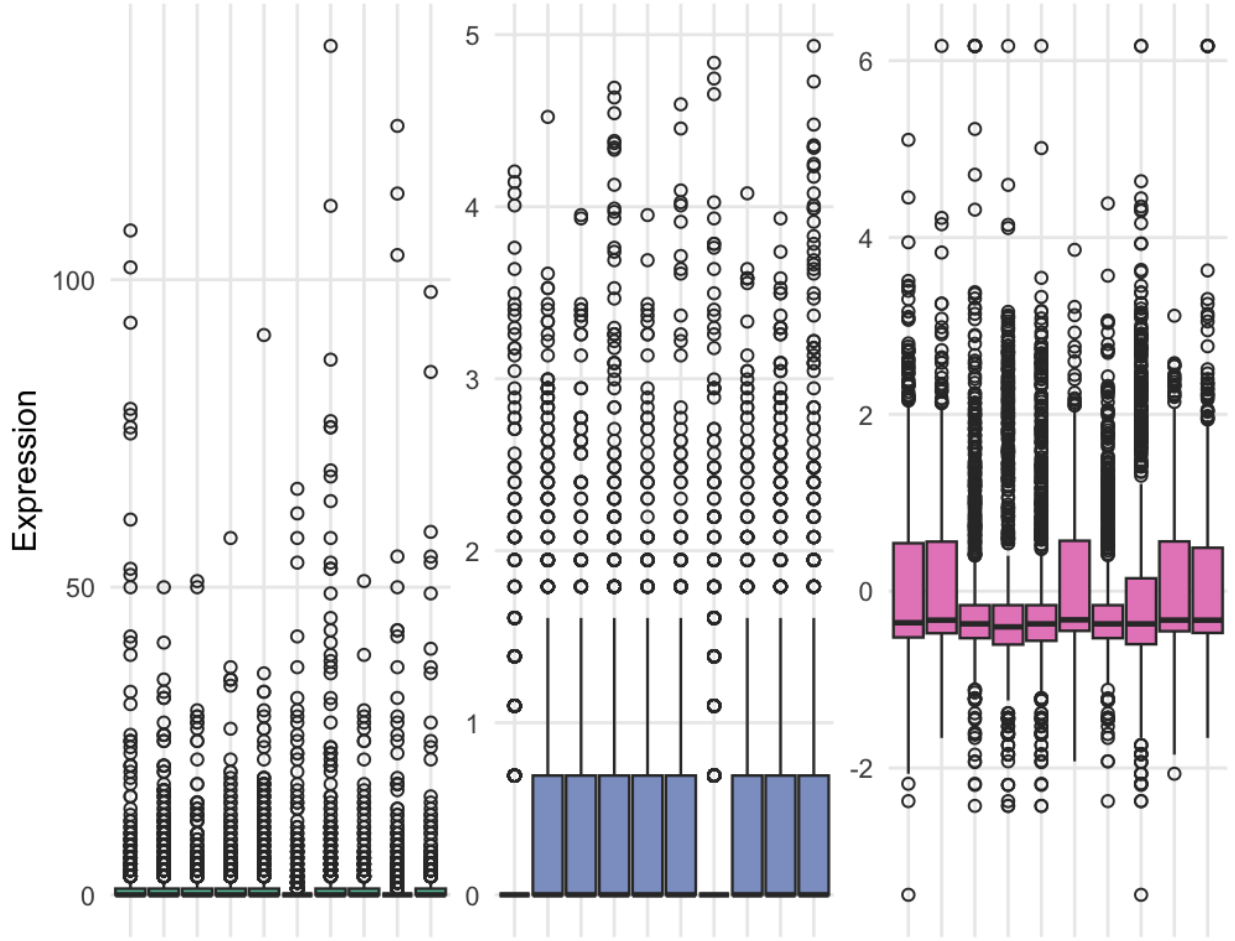
Gene	Mean	SD	Dominates PCA?
LYZ	5	3	YES
CD79A	0.1	0.2	NO

High-expression genes dominate PCA — even if low variance!

Solution: Z-Score Scaling

(Every gene → **mean = 0, SD = 1**)

Scaling



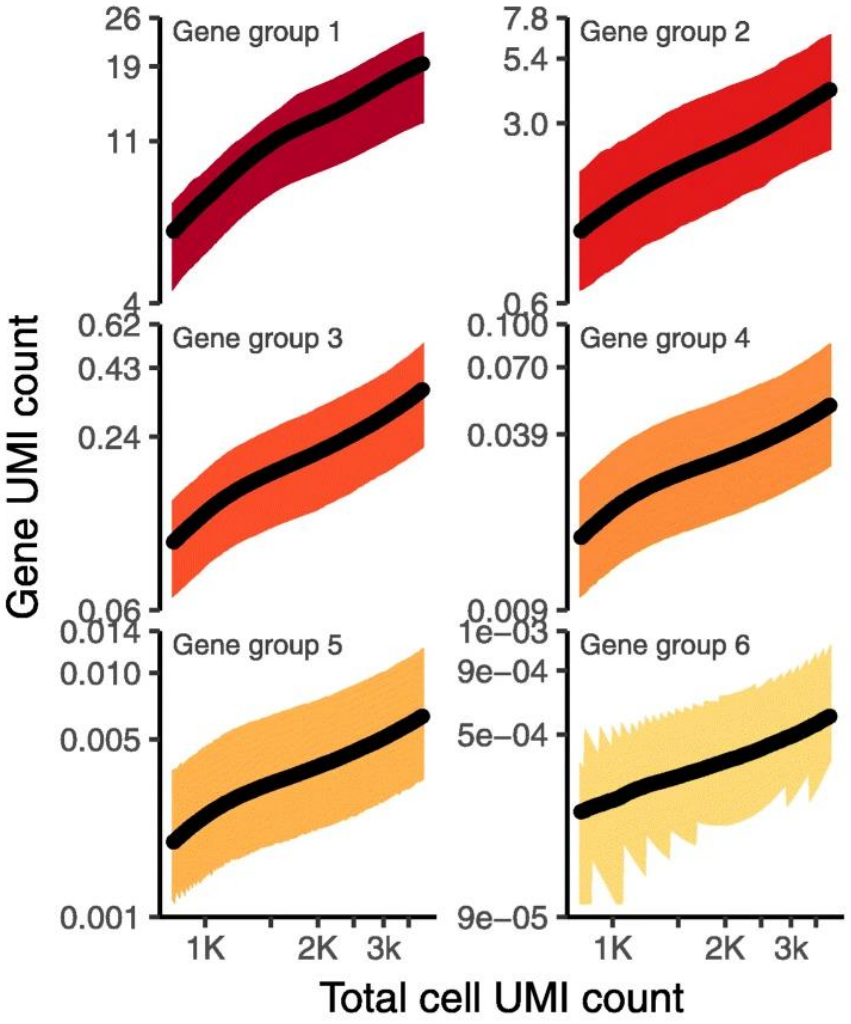
What other transformations can we use?

Does anyone know about `SCTransform`?

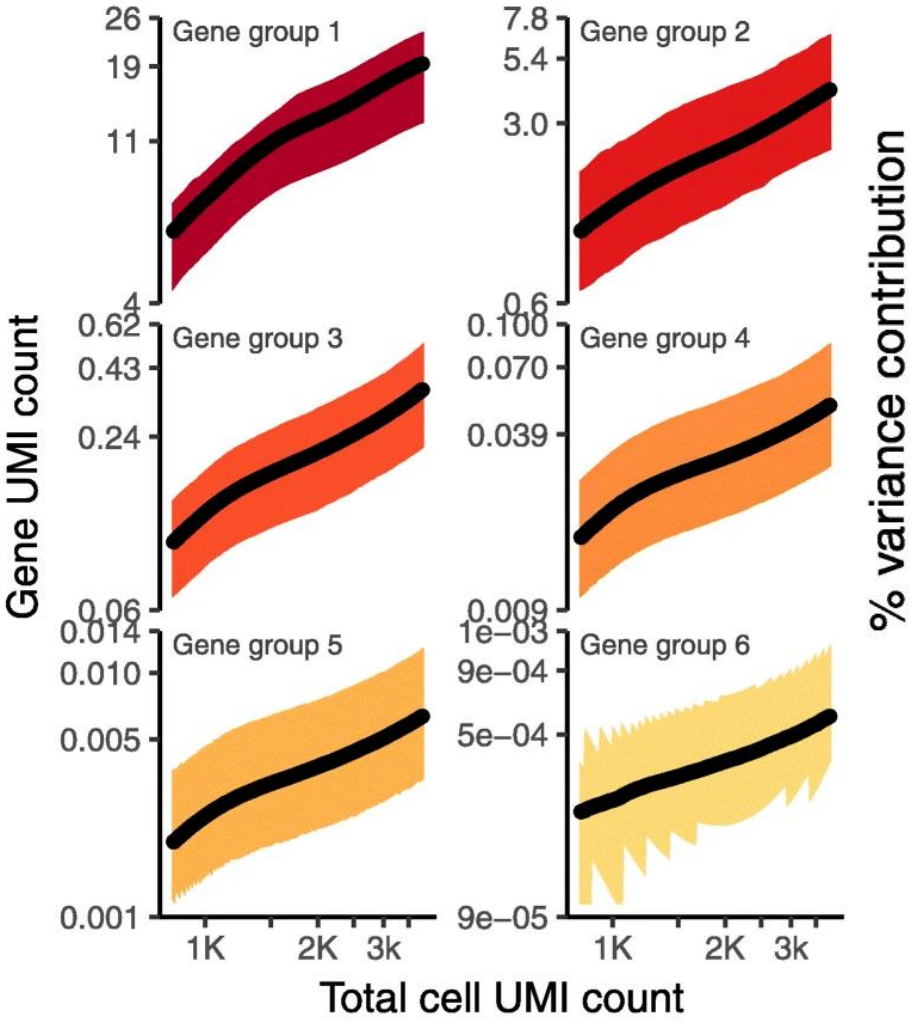
Transformation : Pearson residuals

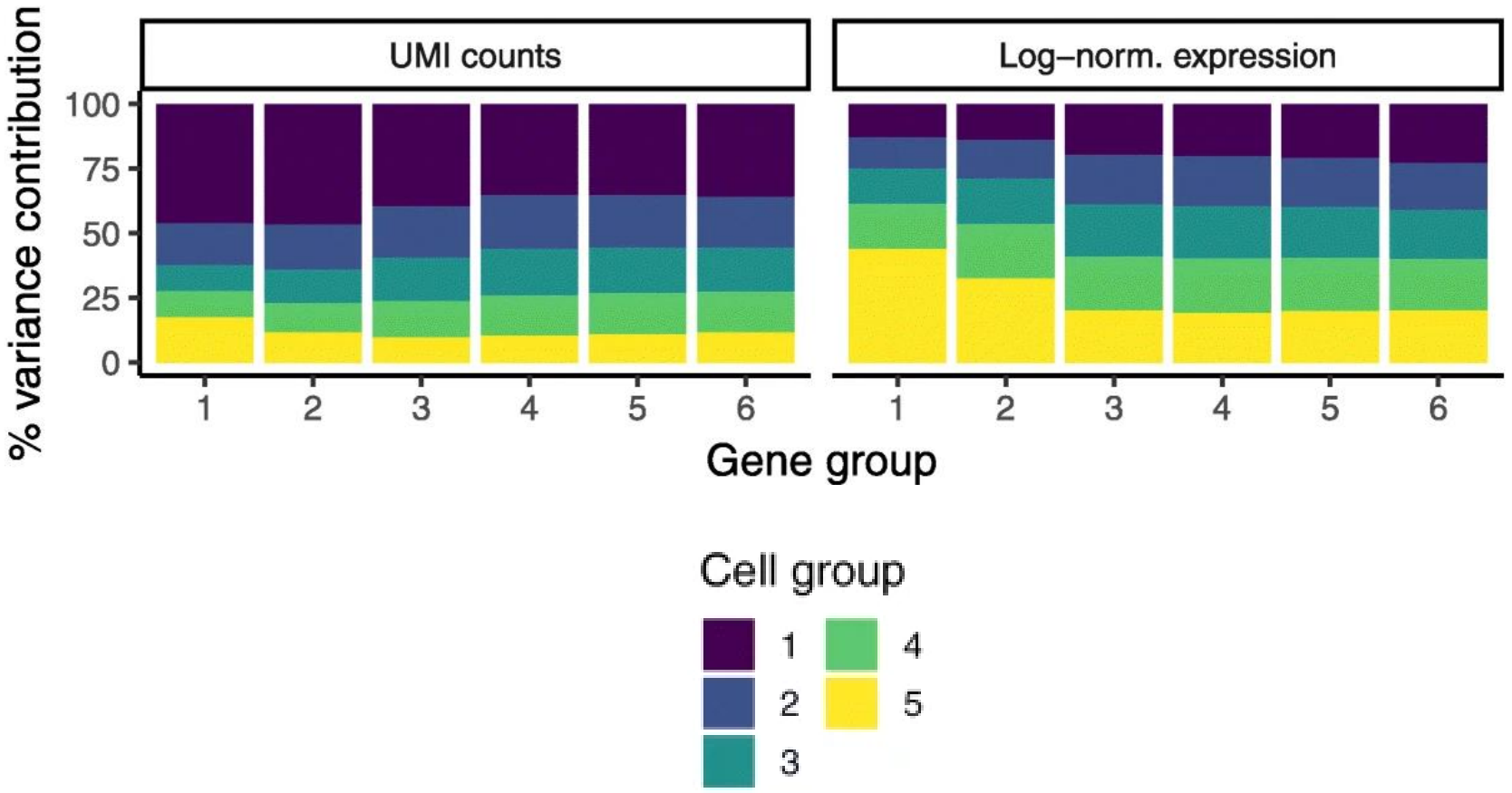
Instead of transforming each measurements individually, Pearson residuals apply a weight to all measurements of a gene

Gene counts are heavily influenced by sequencing depth

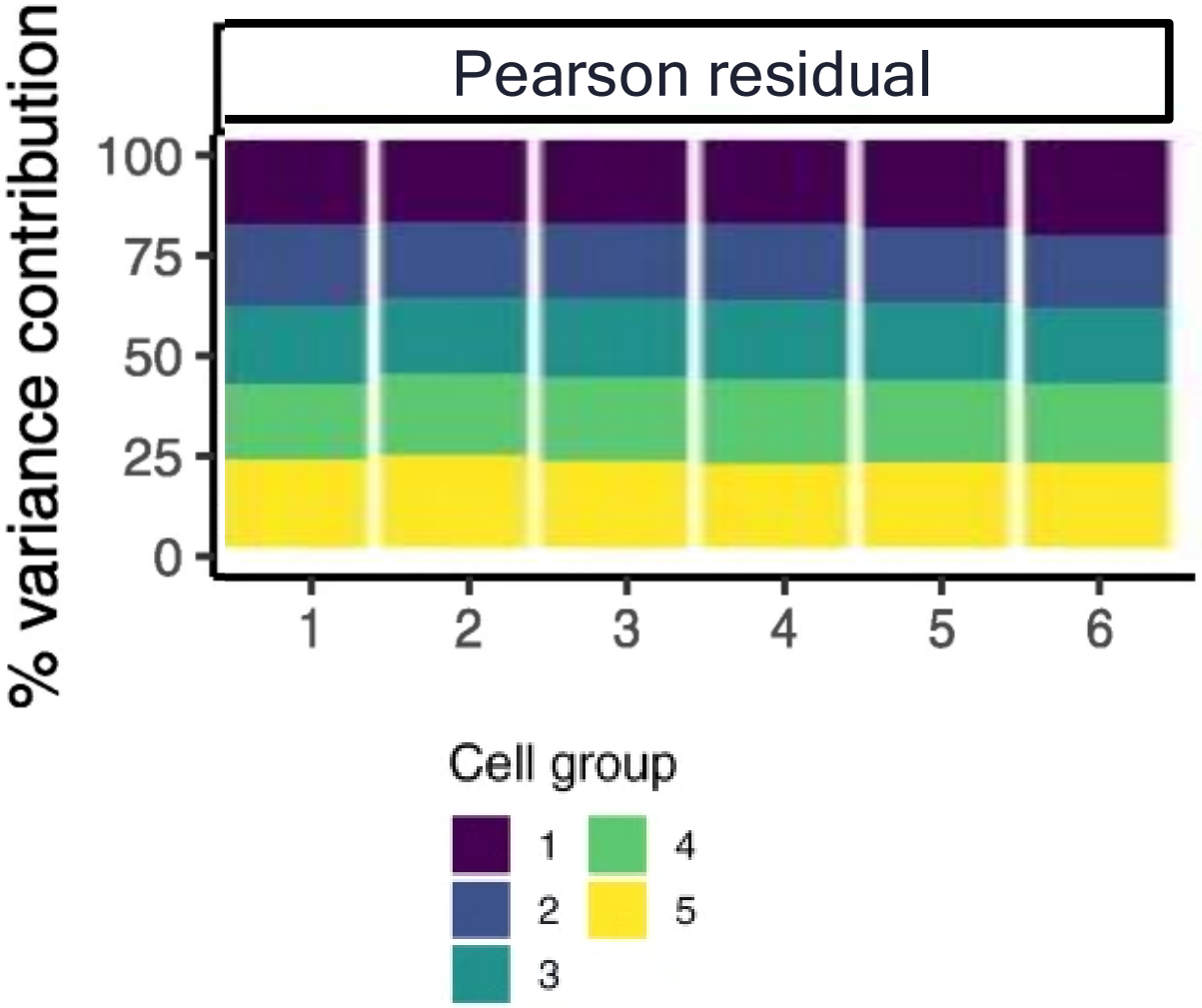


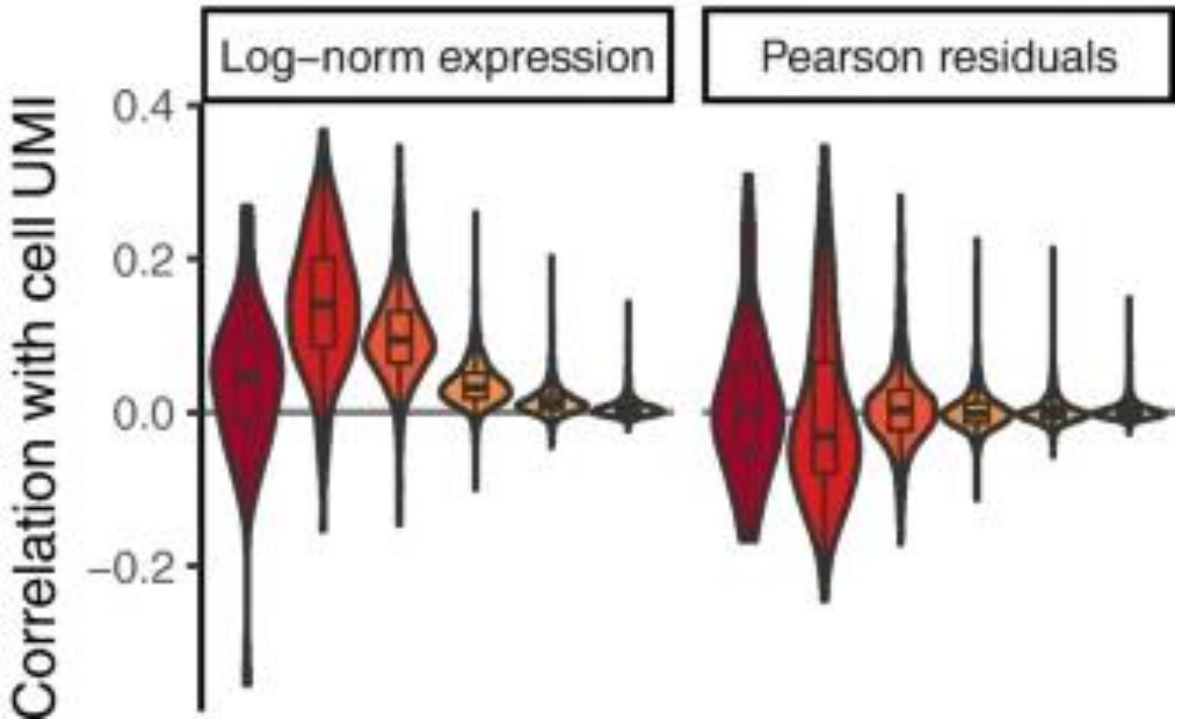
Gene counts are heavily influenced by sequencing depth





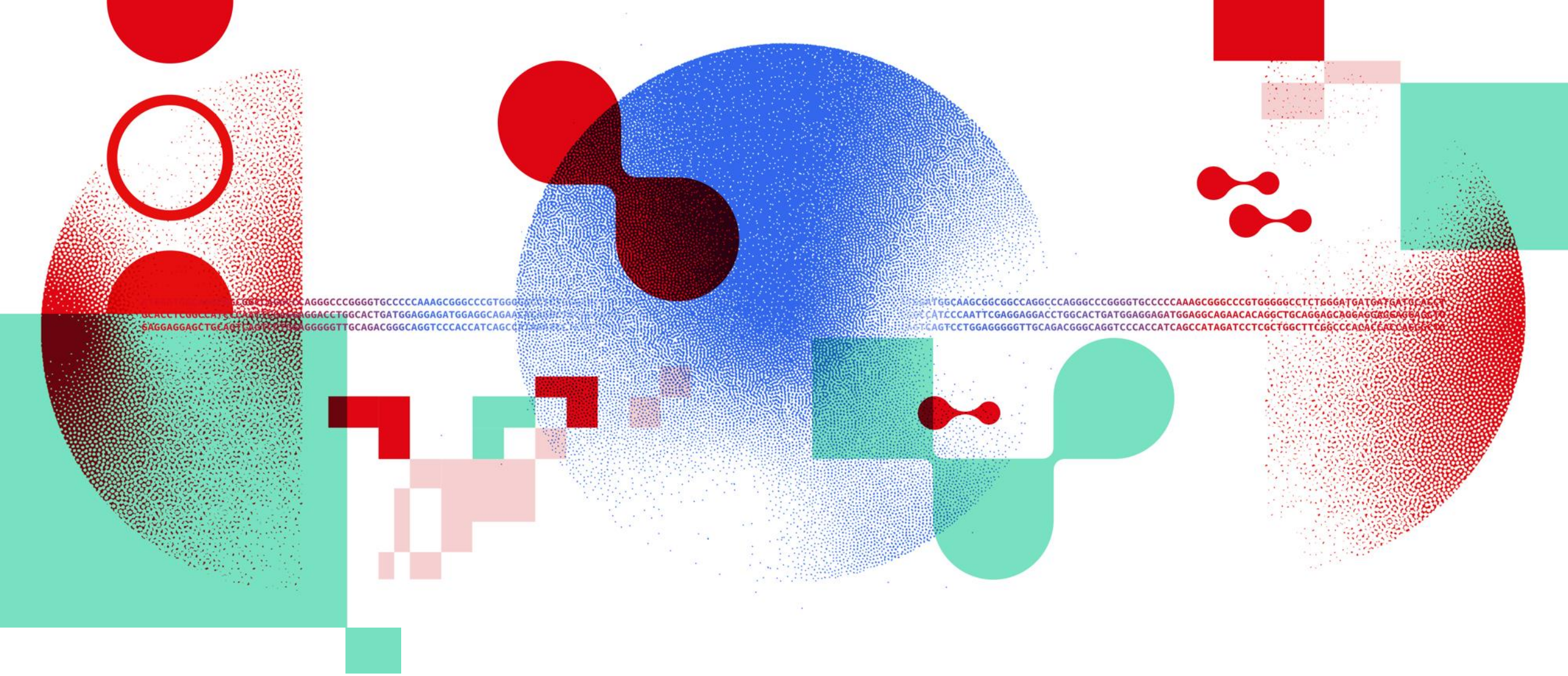
sctransform





We will not use `SCTransform` for the group work due to computation resources. It is advisable to evaluate it for your own single-cell RNA-seq data.

Are scaled values appropriate for downstream analyses like differential expression, and what are the reasons for or against their use?



Thank you

DATA SCIENTISTS FOR LIFE

sib.swiss