

SUMseq: Single-cell Ultra-high throughput Multiome

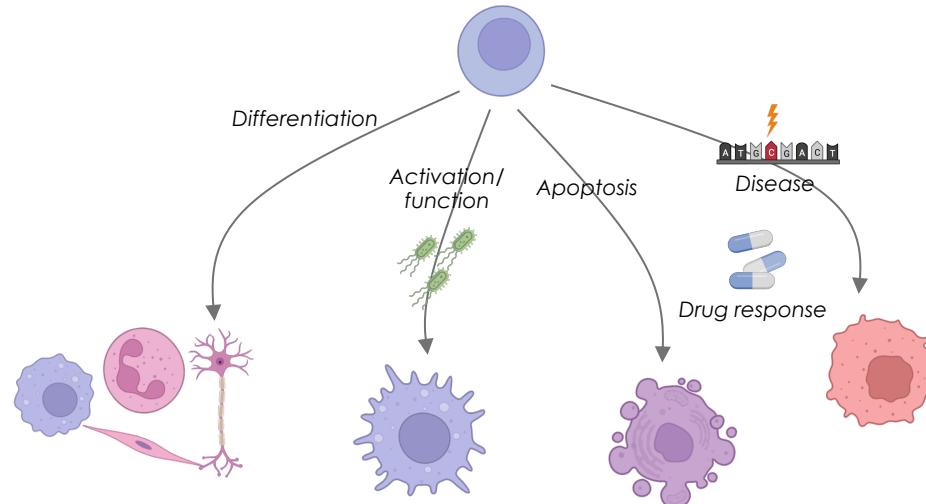
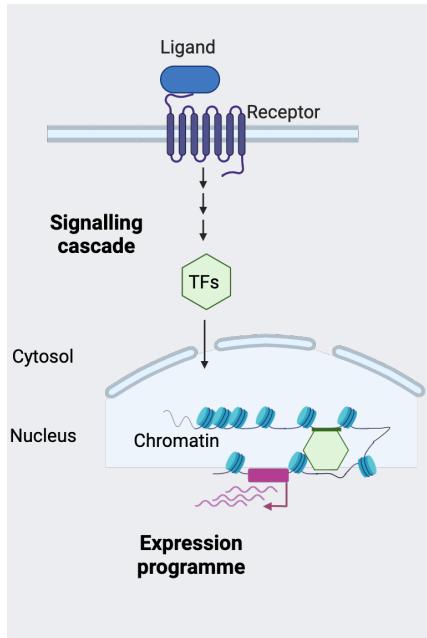
A highly multiplexable and cost-effective single cell RNA/ATAC joint profiling method



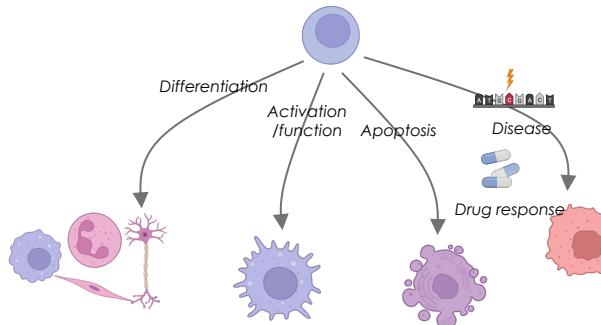
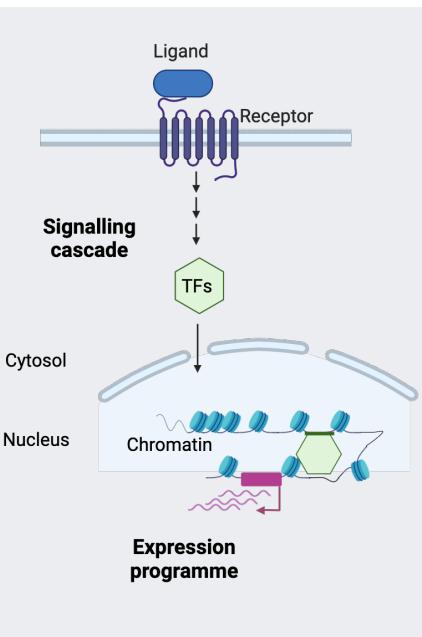
Sara Lobato Moreno

Zaugg group
scATACseq course 2024
12.04.24

Cell differentiation and function are controlled across multiple layers of gene regulation

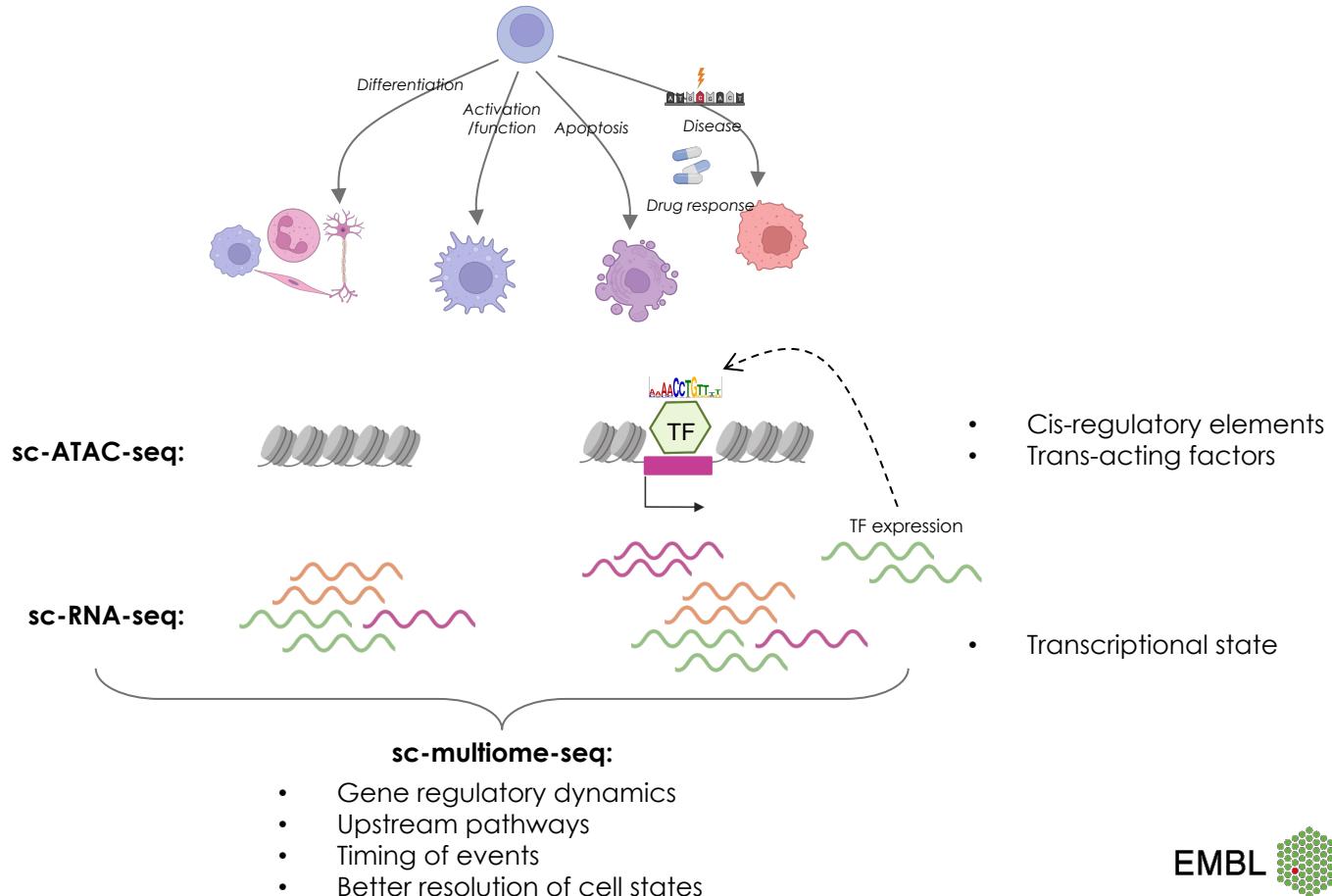
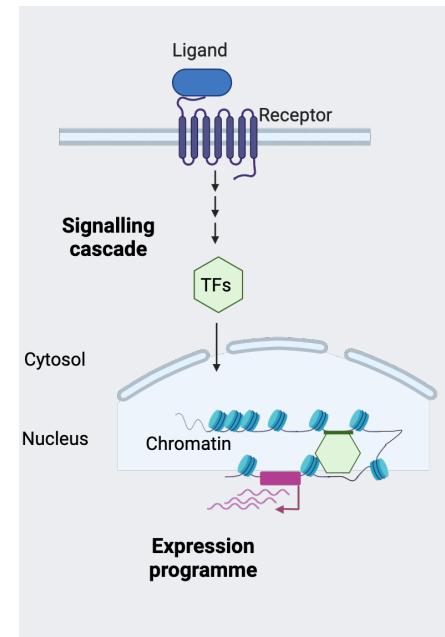


Joint profiling of gene expression and chromatin accessibility enables the exploration of gene regulatory dynamics underlying diverse cellular processes



- Cis-regulatory elements
- Trans-acting factors
- Transcriptional state

Joint profiling of gene expression and chromatin accessibility enables the exploration of gene regulatory dynamics underlying diverse cellular processes



Current limitations of single-cell multiome methods

Cell atlas projects

Cell number scalability

Drug screens

Variability in **human patient material** (need of large cohorts)

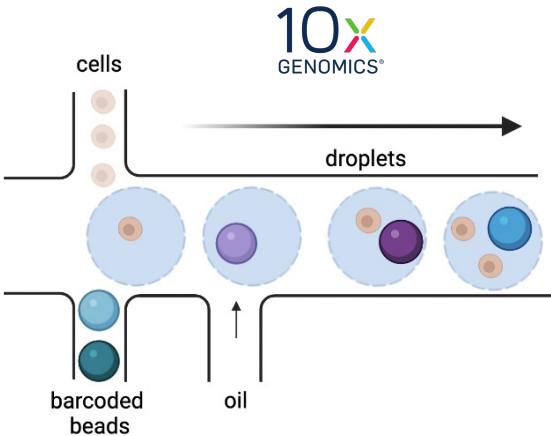
Sample scalability (multiplexing)

Differentiation trajectories (high timepoint resolution)

Costs

Complex tissues

Current limitations of 10x genomics single-cell multiome



Cell number scalability

Maximum ~16.000 nuclei can be loaded per lane to avoid doublets (recovery ~ 60%)

Sample scalability (multiplexing)

Not available for 10x multiome or ATAC kits

Costs

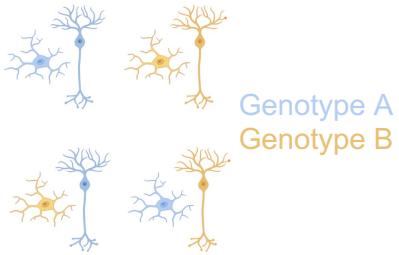
~ 2400 eur/lane (= per sample)

Complex setups require implementing high-throughput approaches

(Cell-type \times genotype) \times

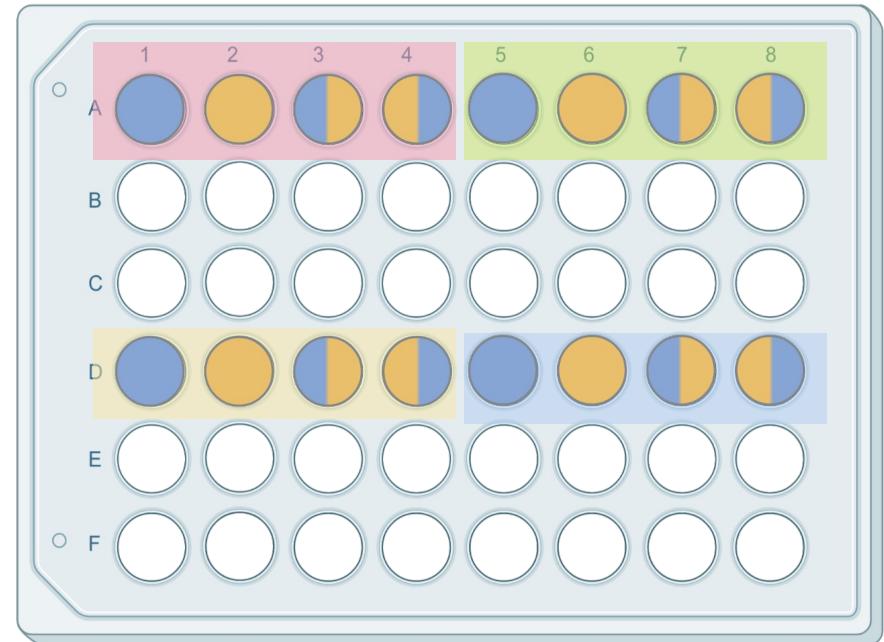
Stimulation

Drug A / drug B / drug C



Basal

Drug A

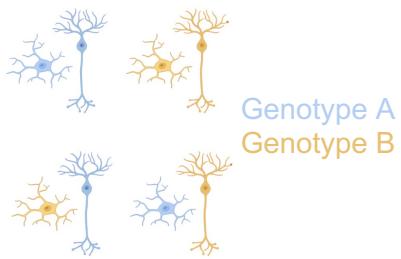


Drug B

Drug C

Complex setups require implementing high-throughput approaches

(Cell-type x genotype) x Stimulation x Time-point



Drug A / drug B / drug C



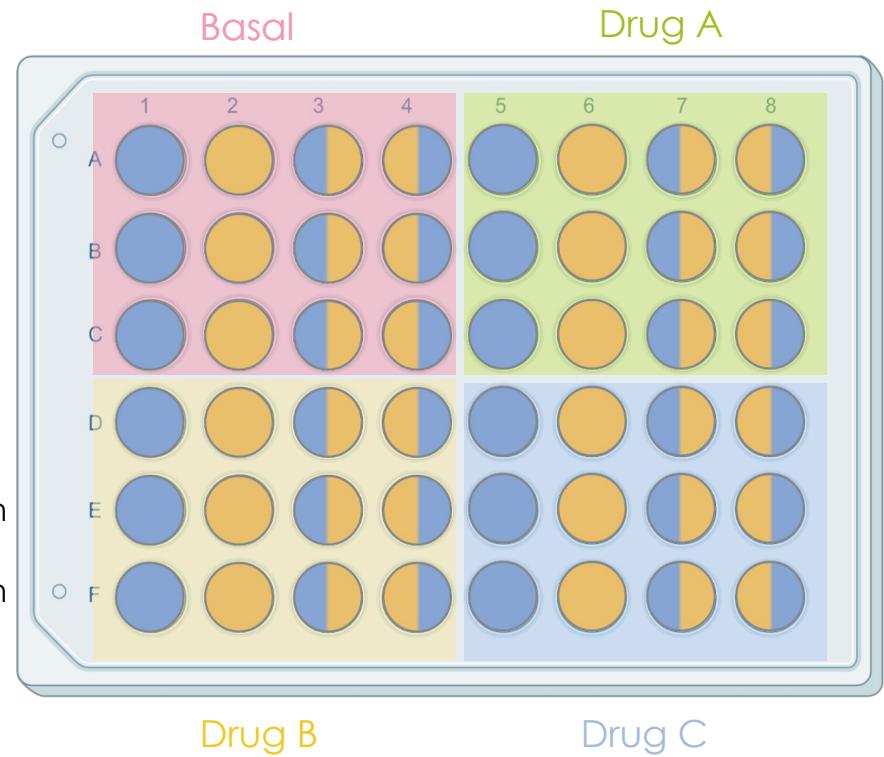
n = 48 samples

10x Genomics multiome
(sc-RNA+ATAC-seq)

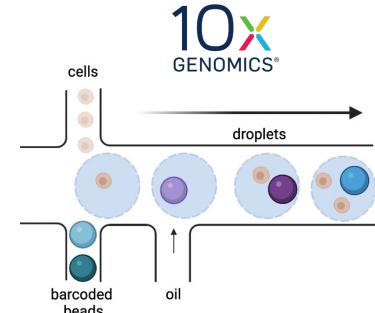
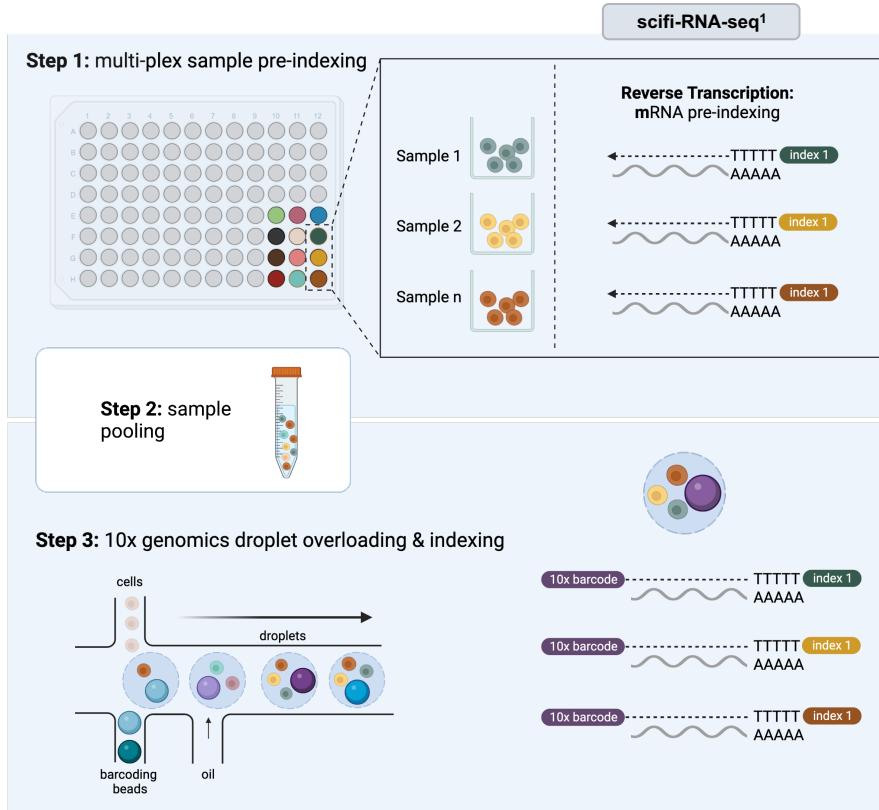
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High-throughput approach required

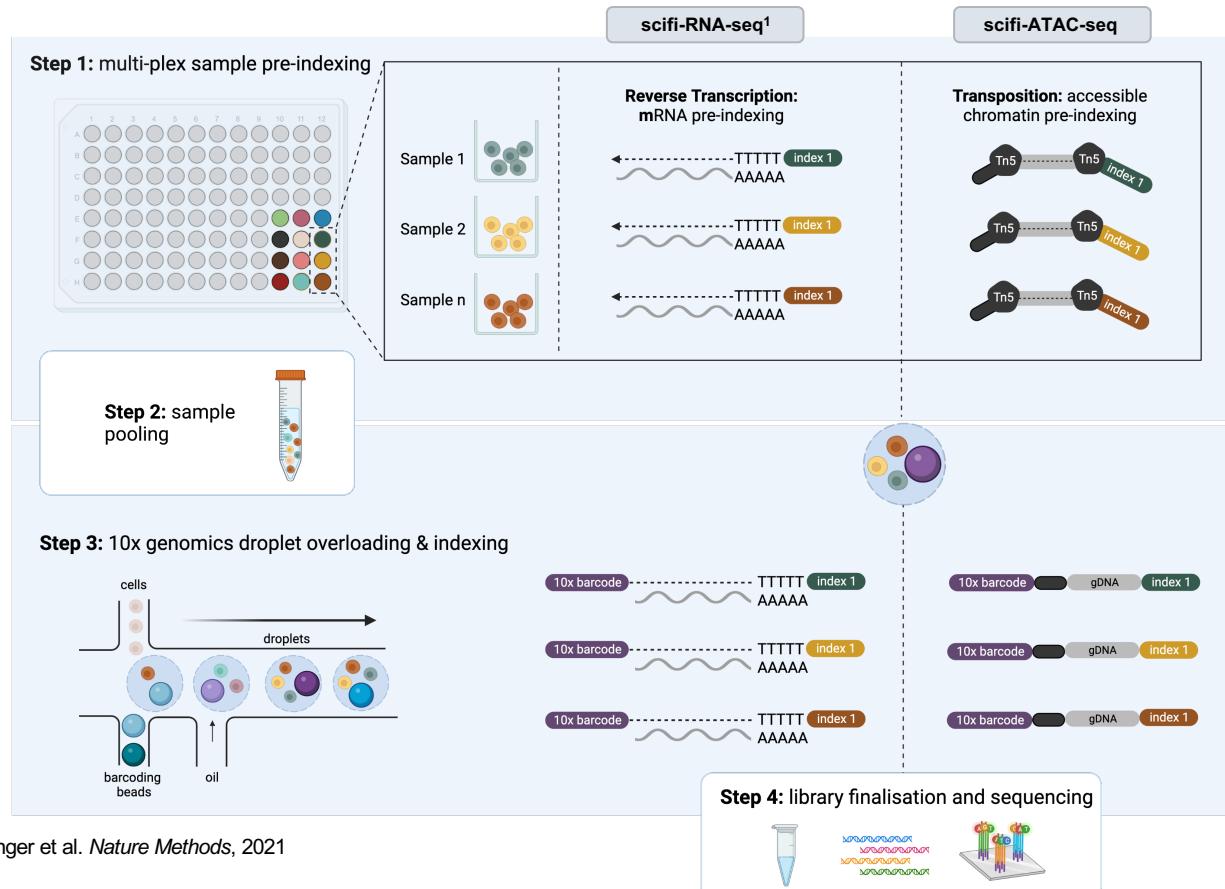
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The principle of single cell combinatorial fluidic indexing ‘scifi’ to maximize the throughput of 10x genomics methods



The principle of single cell combinatorial fluidic indexing ‘scifi’ to maximize the throughput of 10x genomics methods

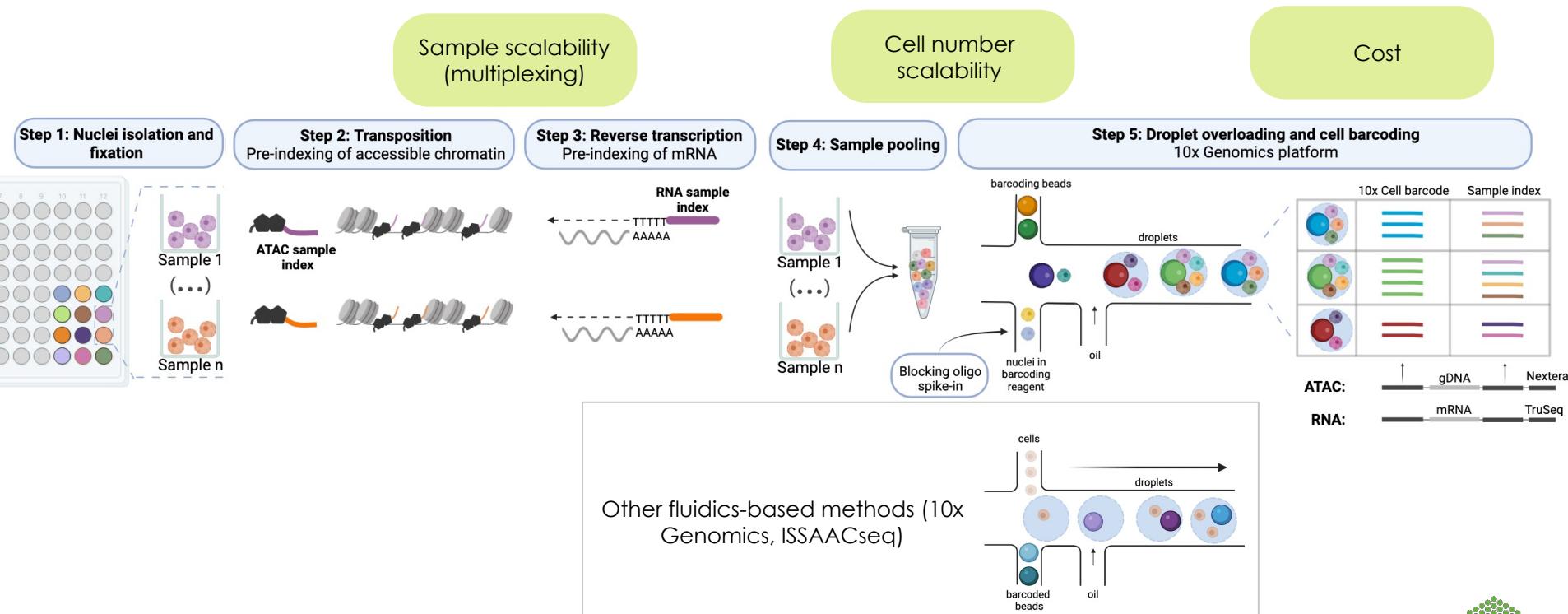


Mikael Marttinen
(Zaugg group)

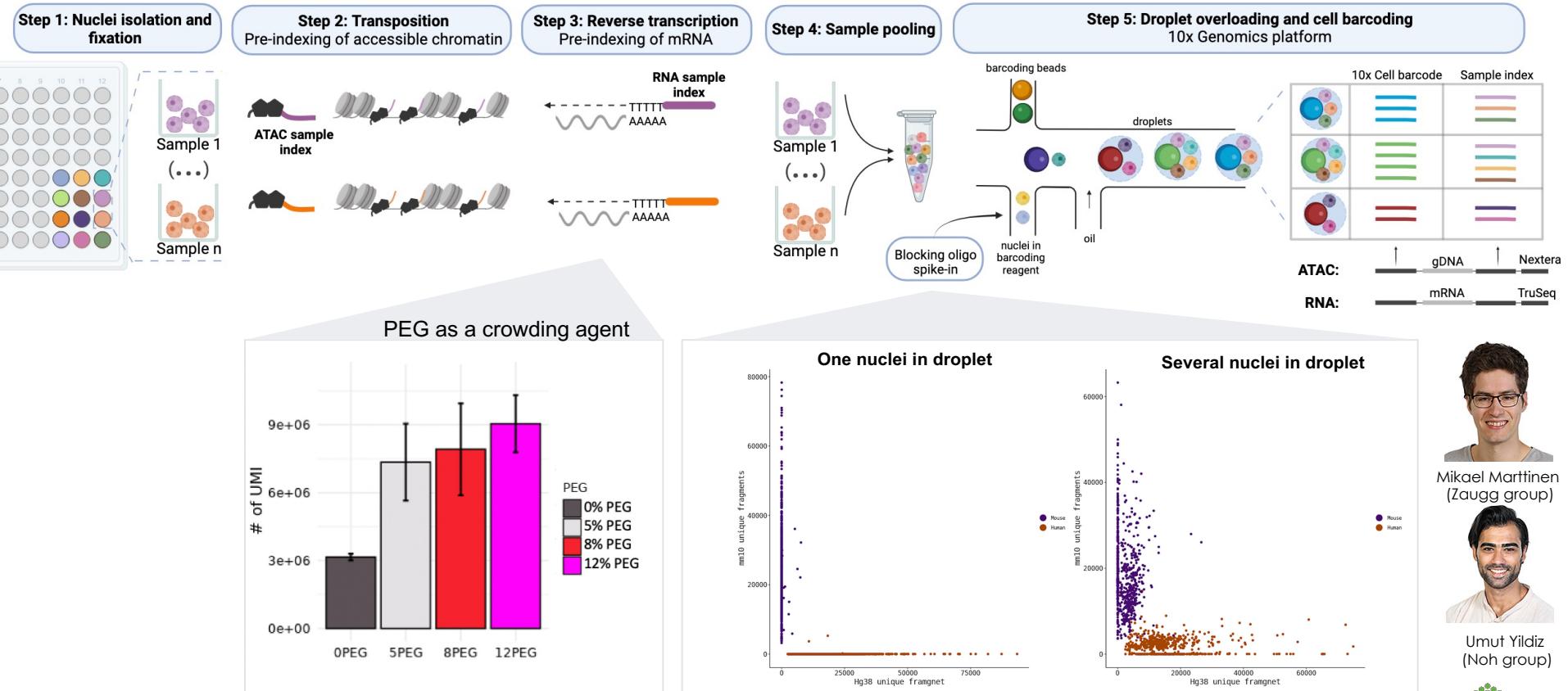


Umut Yıldız
(Noh group)

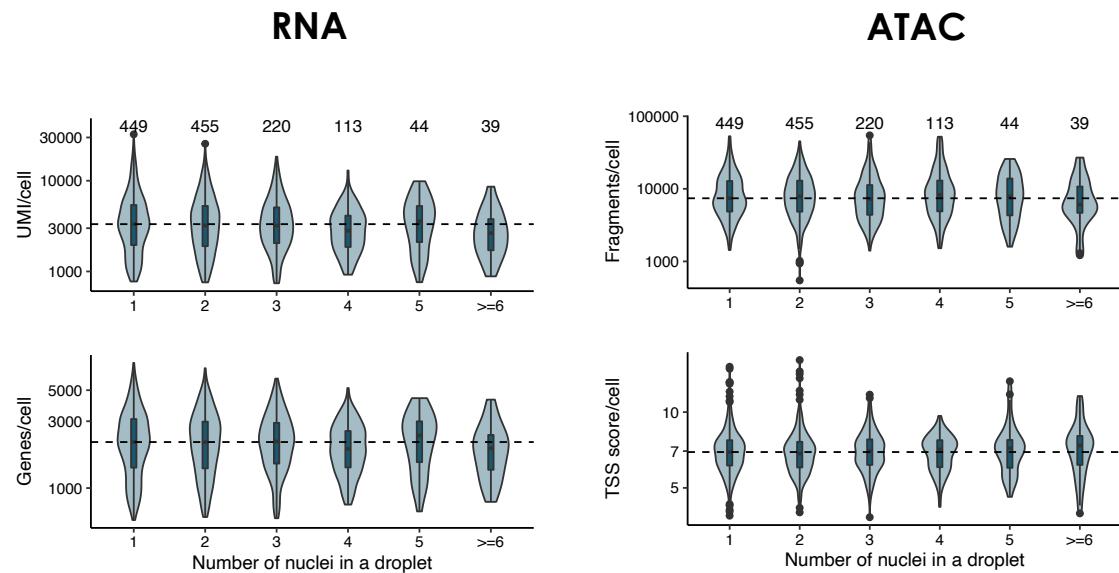
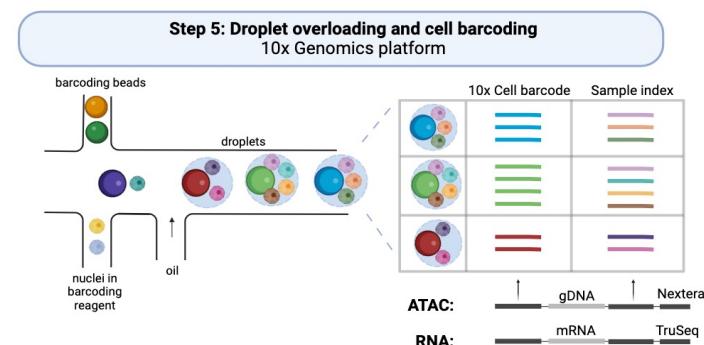
SUMseq (single-cell Ultra-high throughput Multiome) addresses main limitations



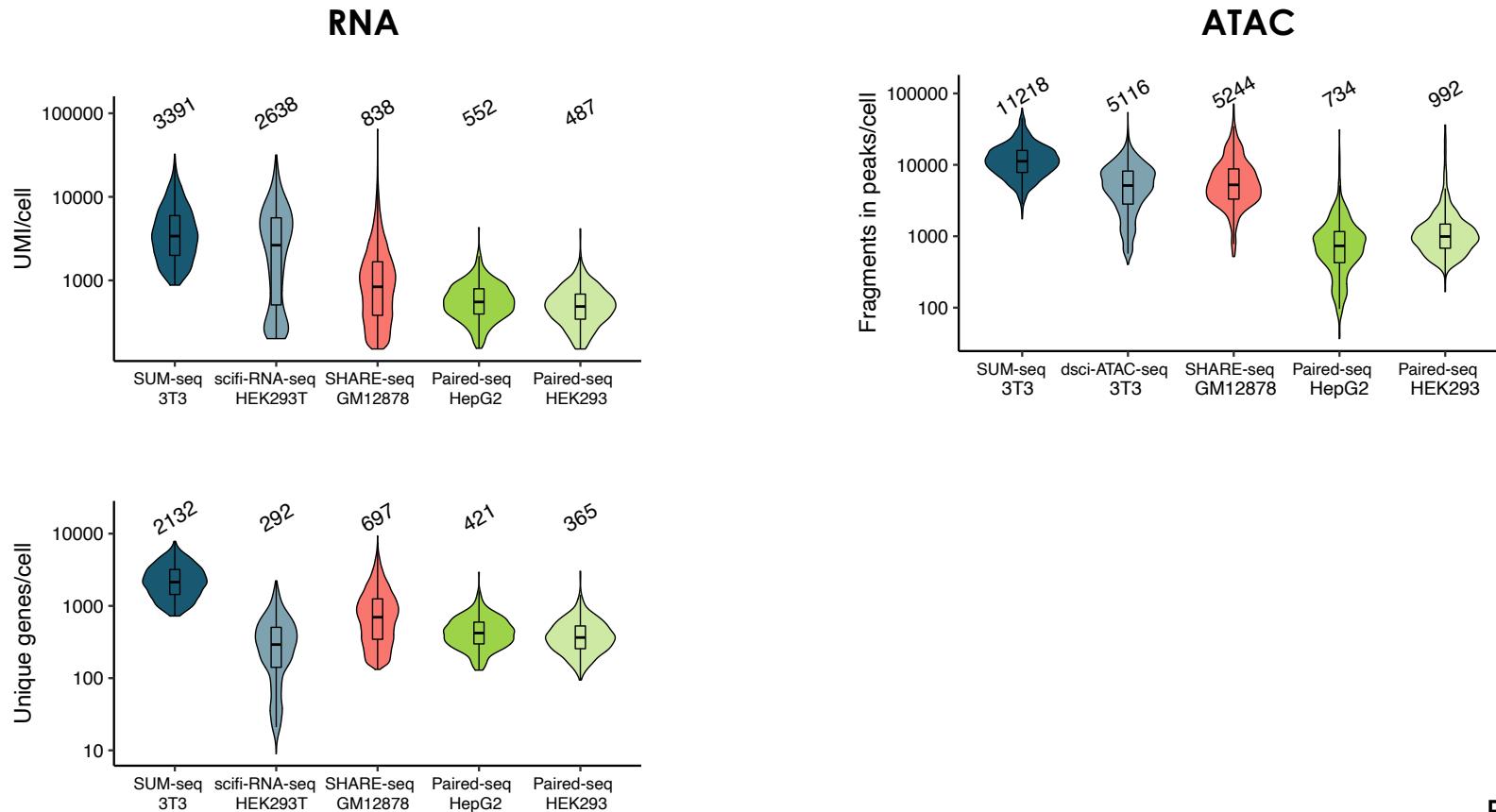
Some of the key optimization steps applied to SUMseq



Droplet overloading does not have an impact on data complexity



SUMseq quality metrics and comparison with other sc-multiome methods



The importance of sample preparation

It is crucial to optimize it for every sample type! This part should not be underestimated

Nuclei isolation

- Nuclei isolation or cell permeabilization
- Detergent concentration
- Lysis time

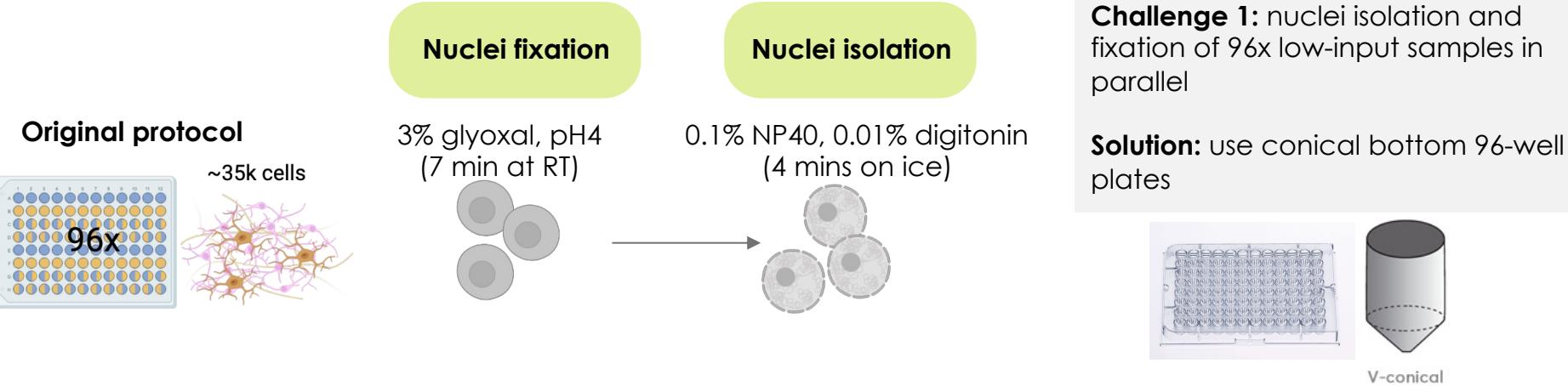
Nuclei fixation

- Fixative to use (PFA, glyoxal, DSP)
- Fixative concentration and pH (*can have a huge impact!*)
- Fixation time

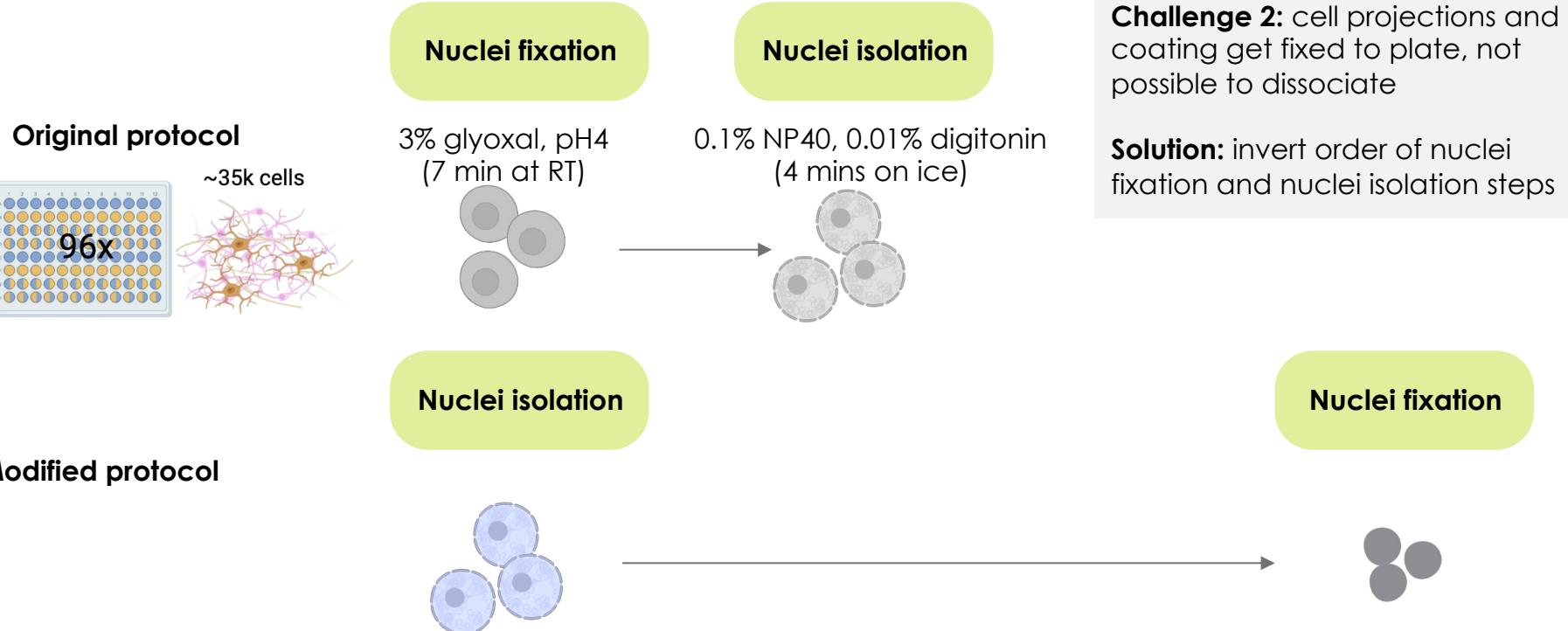
Debris removal

- Strainers (mash strainer, flowmi)
- Sucrose gradient
- Glycerol buffer

The importance of sample preparation: a real life example

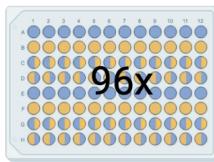


The importance of sample preparation: a real life example

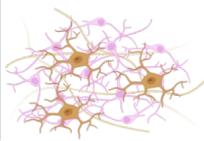


The importance of sample preparation: a real life example

Original protocol

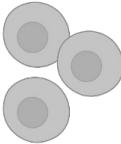


~35k cells



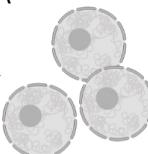
Nuclei fixation

3% glyoxal, pH4
(7 min at RT)



Nuclei isolation

0.1% NP40, 0.01% digitonin
(4 mins on ice)

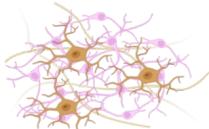
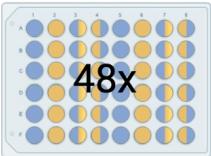


Challenge 3: Recovery per sample too low

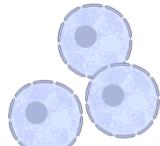
Solution: up-scale to 48-well format and increase cell number

Modified protocol

~120k cells



Nuclei isolation

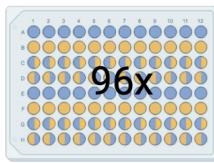


Nuclei fixation

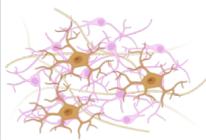


The importance of sample preparation: a real life example

Original protocol

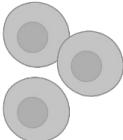


~35k cells



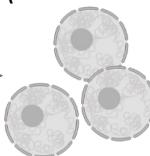
Nuclei fixation

3% glyoxal, pH4
(7 min at RT)



Nuclei isolation

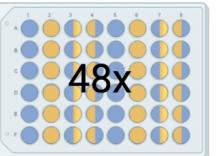
0.1% NP40, 0.01% digitonin
(4 mins on ice)



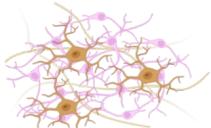
Challenge 4: Too much debris, high risk of 10x chromium clogging

Solution: add filtering steps

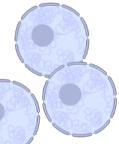
Modified protocol



~120k cells

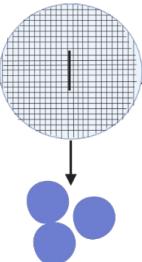


Nuclei isolation



Debris removal

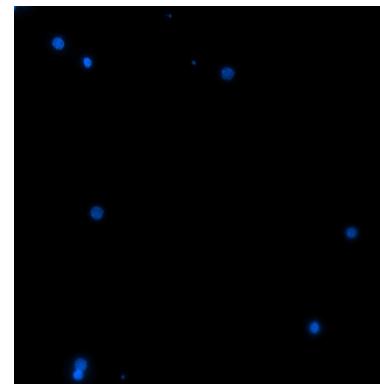
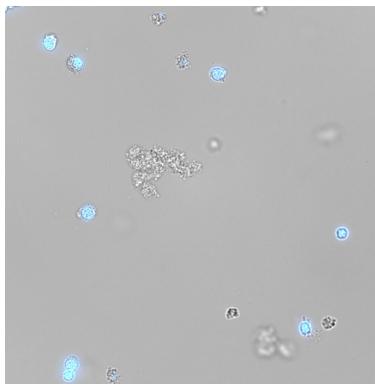
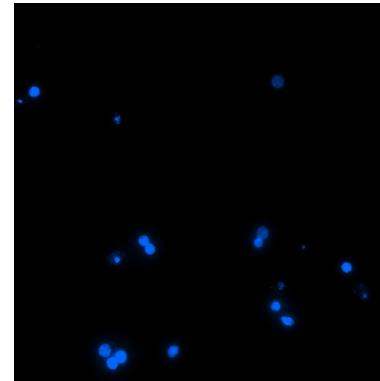
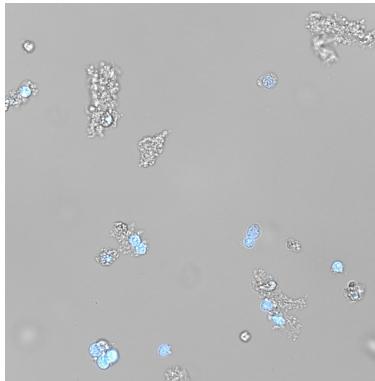
96-plex 40 μ M strainer



Nuclei fixation

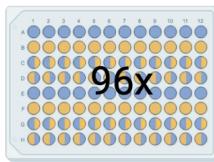


Example of nuclei prior 10x chromium loading, debris present

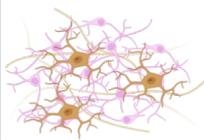


The importance of sample preparation: a real life example

Original protocol

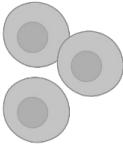


~35k cells



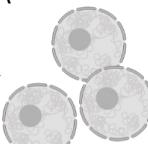
Nuclei fixation

3% glyoxal, pH4
(7 min at RT)



Nuclei isolation

0.1% NP40, 0.01% digitonin
(4 mins on ice)

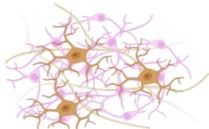
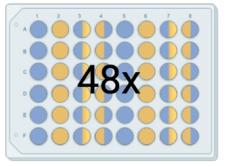


Challenge 5: overlysed nuclei

Solution: reduce detergent concentrations and lysis time

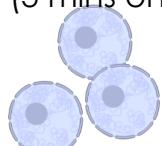
Modified protocol

~120k cells



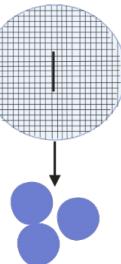
Nuclei isolation

0.025% NP40, 0.001% digitonin
(3 mins on ice)



Debris removal

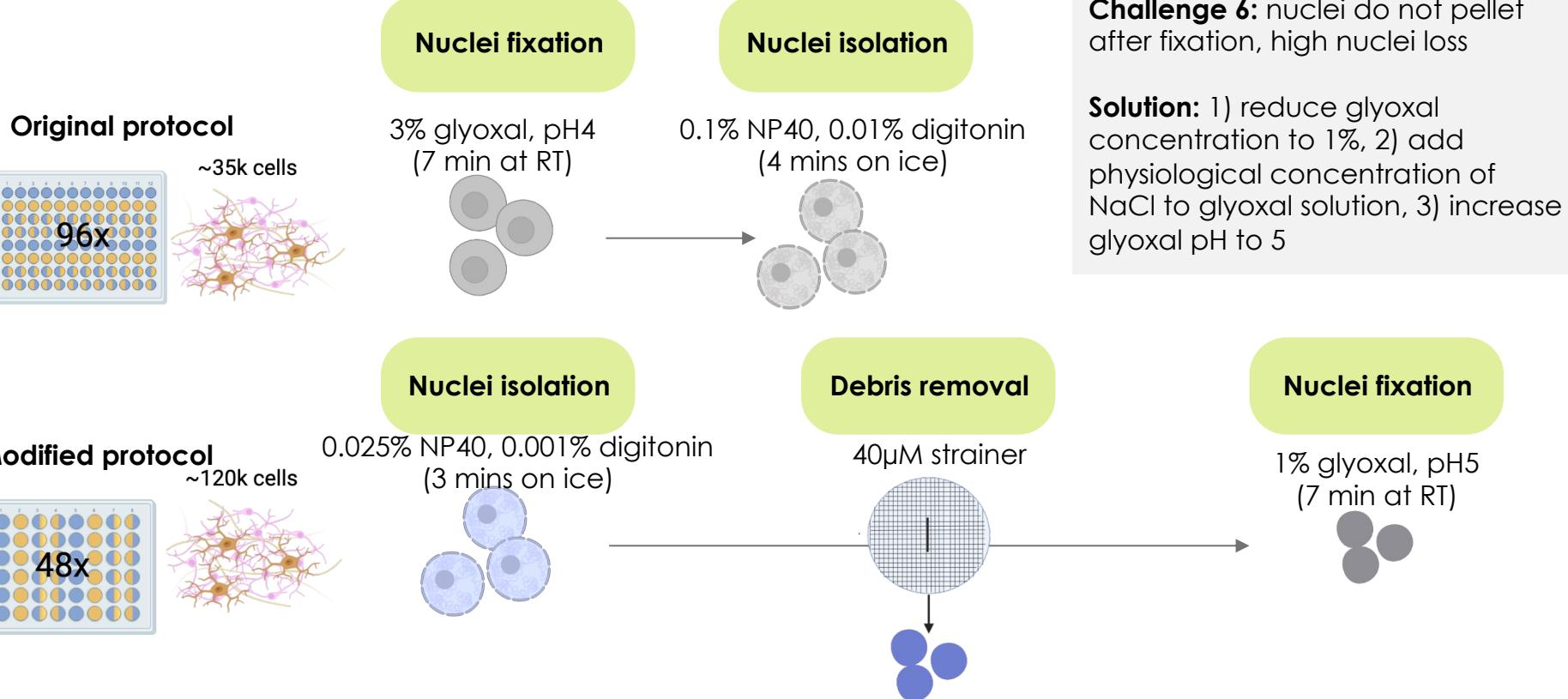
40 μ M strainer



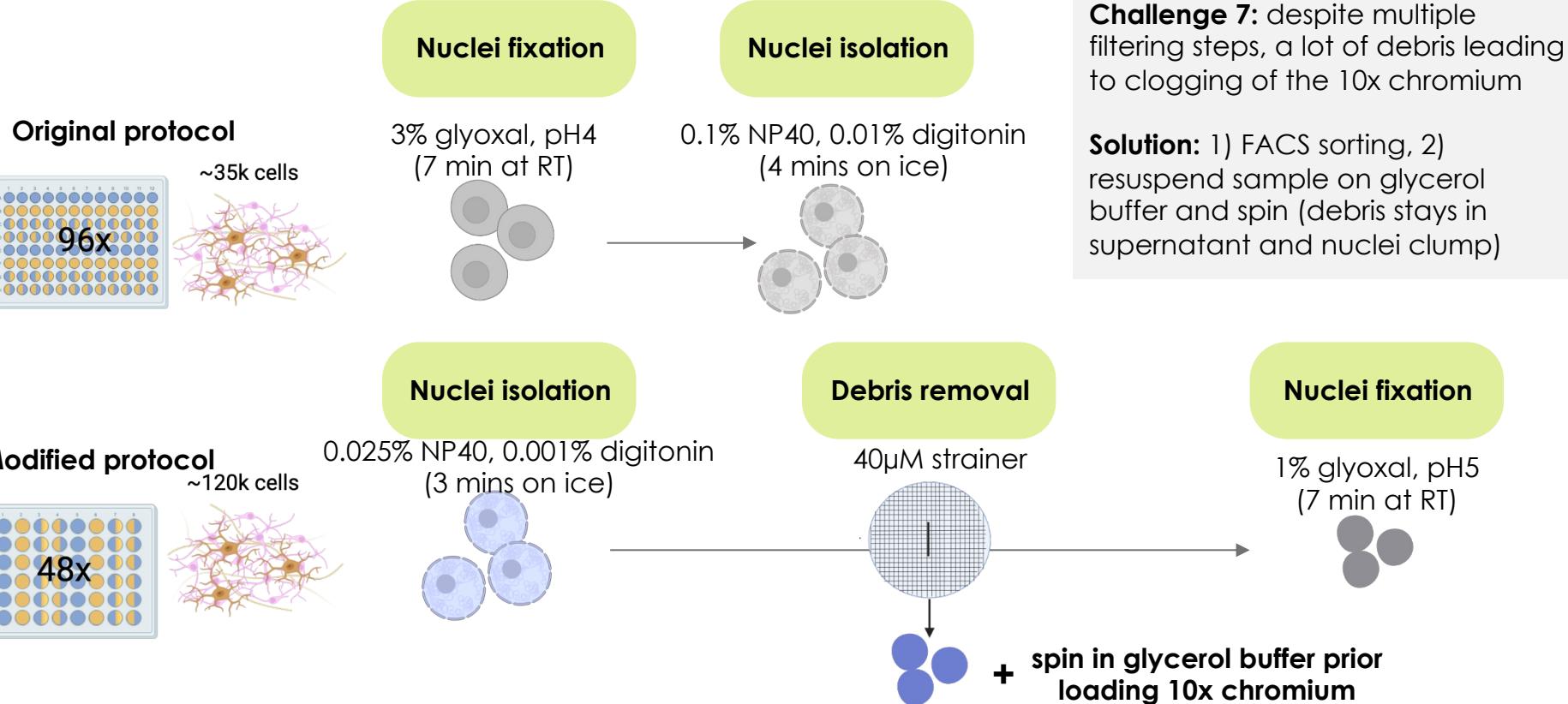
Nuclei fixation



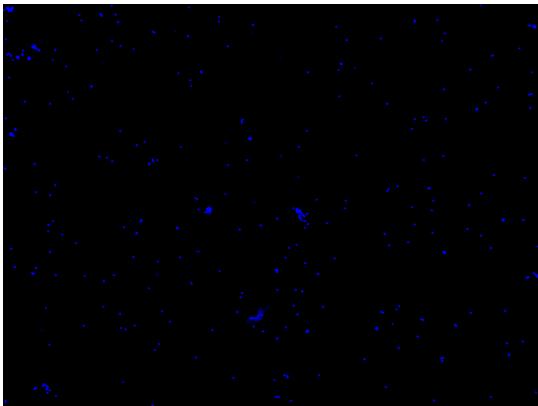
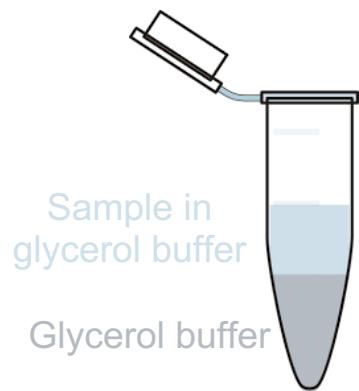
The importance of sample preparation: a real life example



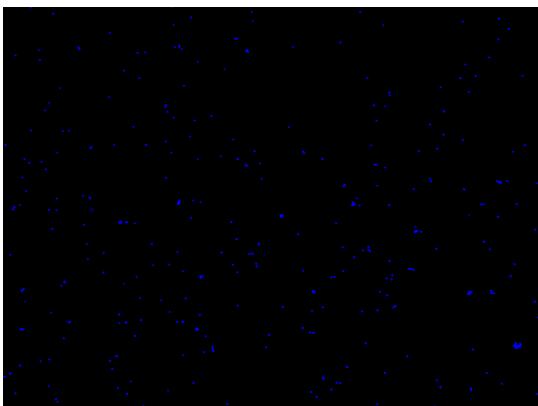
The importance of sample preparation: a real life example



Example of nuclei with and without debris clearance in glycerol buffer



**Before glycerol
buffer spin**



**After glycerol
buffer spin**

Summary

- Profiling mRNA expression and chromatin accessibility from single cells allows to study gene regulatory programmes driving cell differentiation and function in health and disease.
- SUMseq is a ultra-high throughput single-cell multiome method that allows profiling hundreds of thousands of cells and hundreds of samples at reduced costs.
- Sample preparation is a key step when performing single-cell methods. The time required to implement the ideal protocol for your sample of interest should not be underestimated, especially when using complex sample types.