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# Doing more with less: comparative analysis of sample multiplexing methods for single-cell RNA-seq

Viacheslav Mylka

VIB

29.06.2023

# VIB-Janssen Single Cell Tech Collaboration

**Aim:**

Join forces to
test and implement emerging single cell technologies

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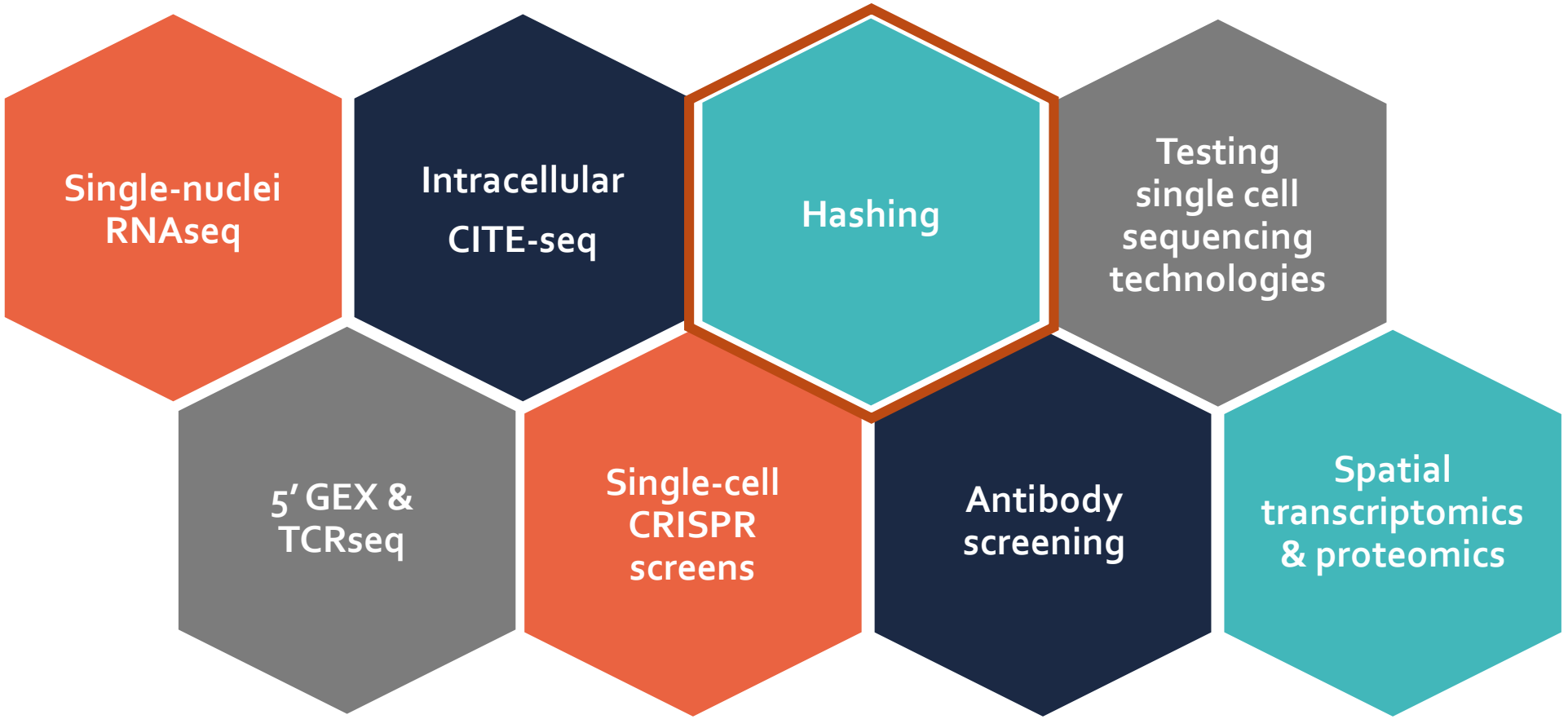
# Project Plan



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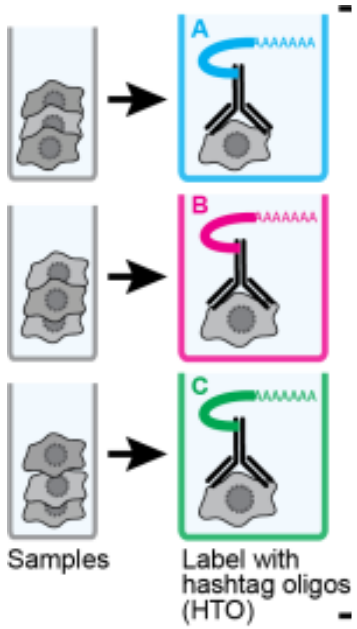
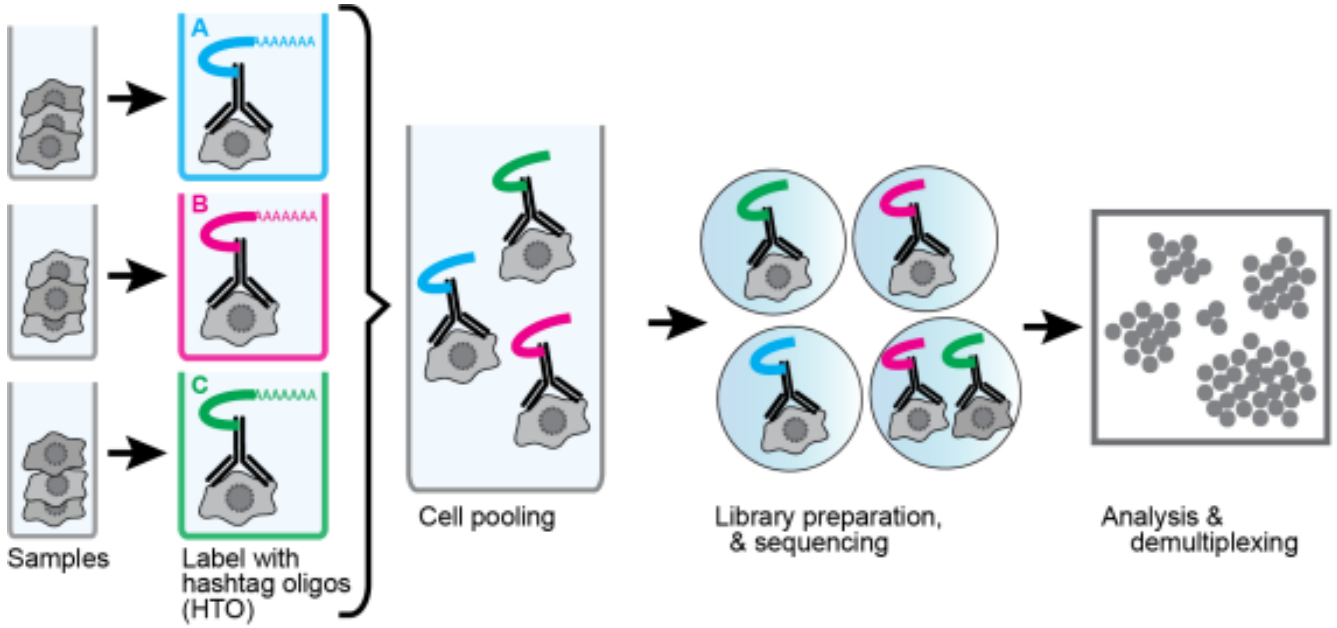
# Agenda

1. Introduction
2. Evaluation of different hashing strategies on human cells and nuclei, including PBMCs from clinical trials
3. Hashing of primary mice tissues
4. Conclusions

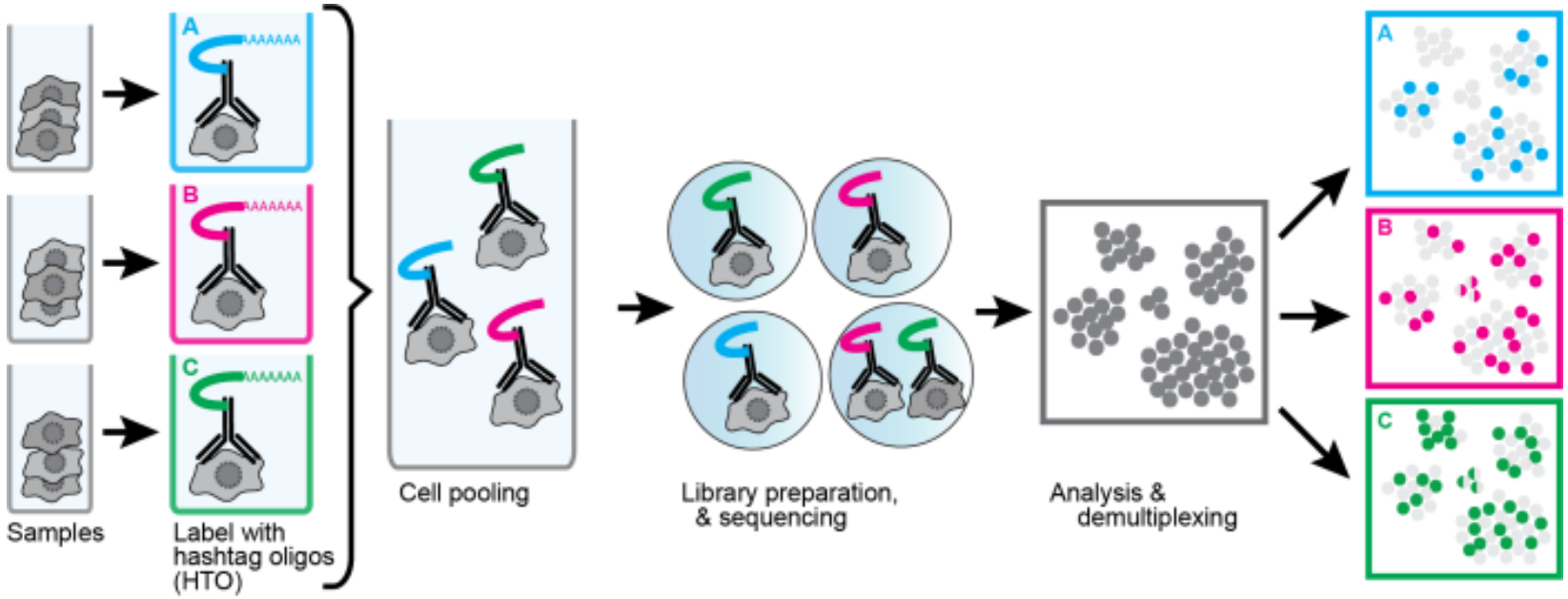
# Sample multiplexing for scRNA-seq



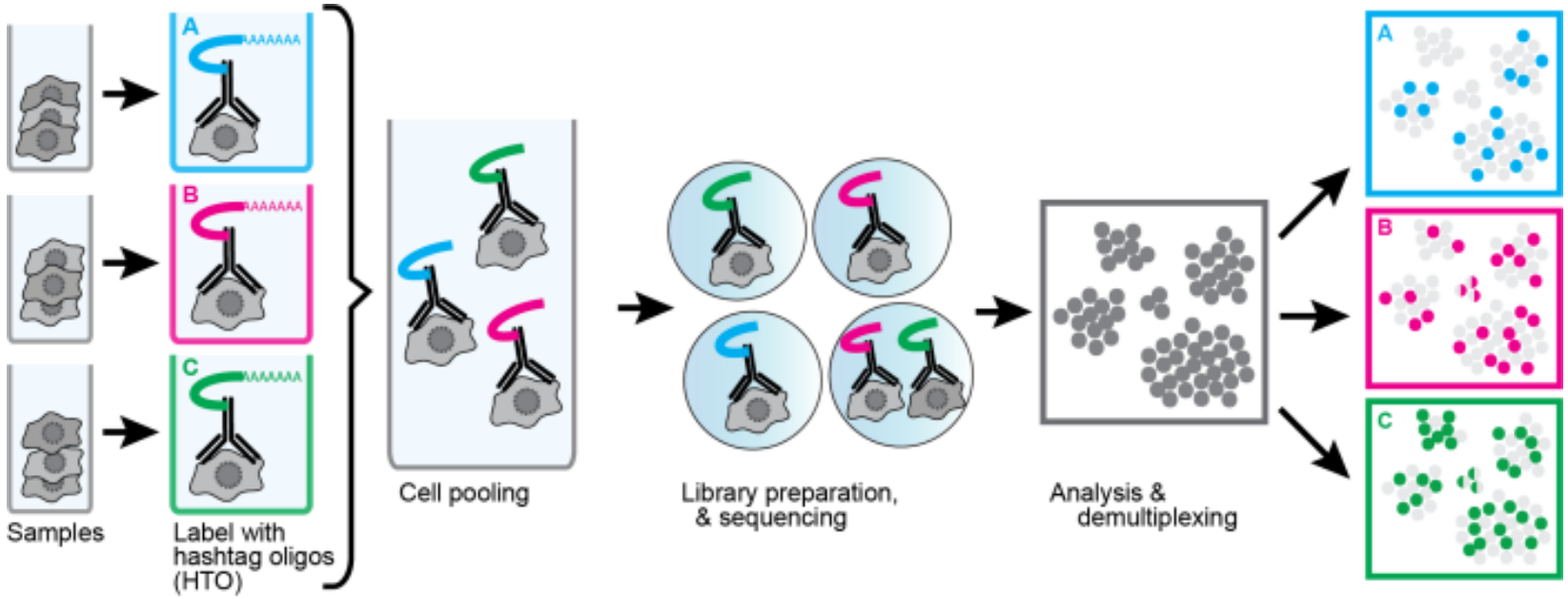
# Sample multiplexing for scRNA-seq



# Sample multiplexing for scRNA-seq



# Sample multiplexing for scRNA-seq



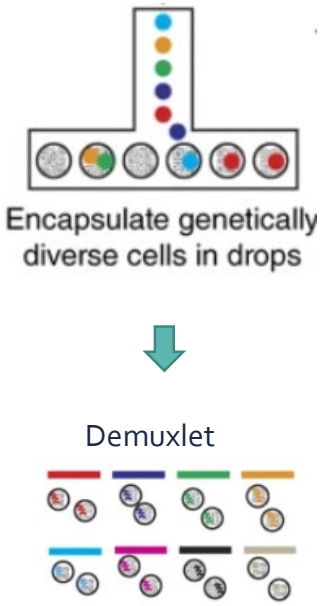

**Advantages of hashing:**

- Diminishing batch effects
- Cost reduction
- Extra layer of data (hashes) for doublet detection

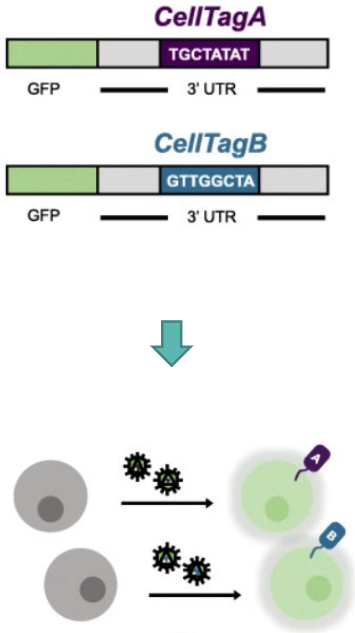
# Landscape of hashing technologies

| Antibody based | Genetic | | Direct labelling | |
|---|---|---|---|---|
| Totalseq Biolegend, AbSeq BD | Pre-existing variation | CellTag indexing | iEDDA click chemistry | MULTIseq lipid conjugation |

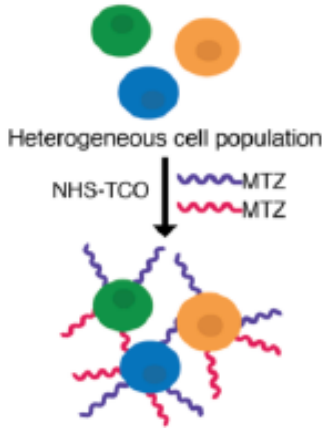
Stoeckius et al. 2018
Gaublomme et al. 2019



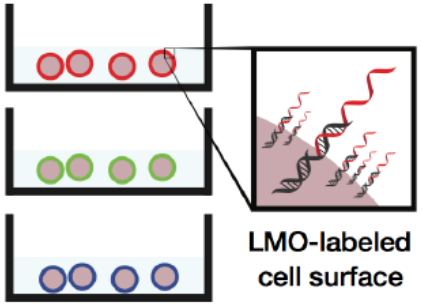

Kang et al. 2018

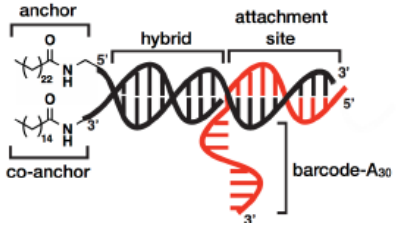



Guo et al. 2019



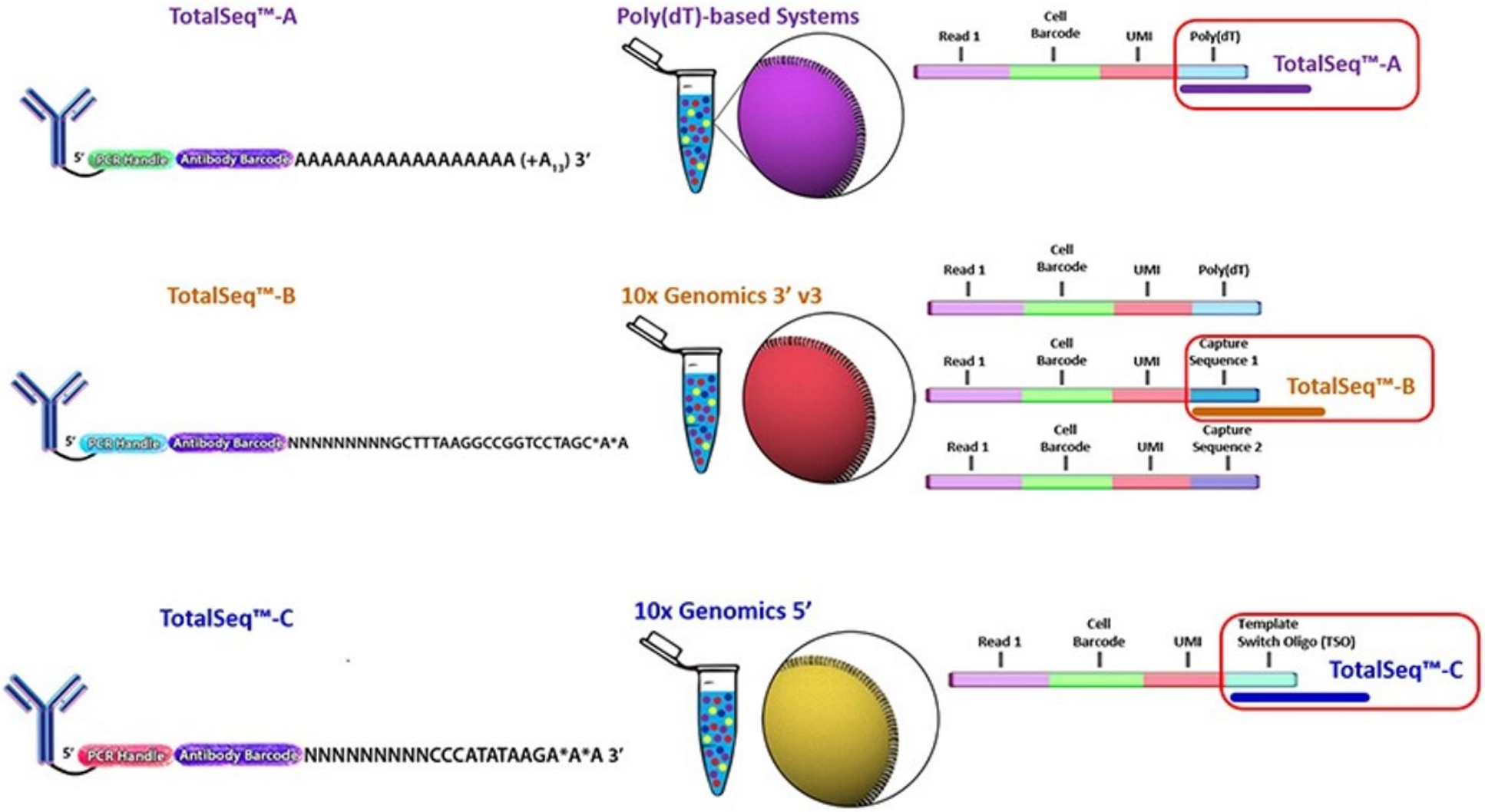

Gehring et al. 2018



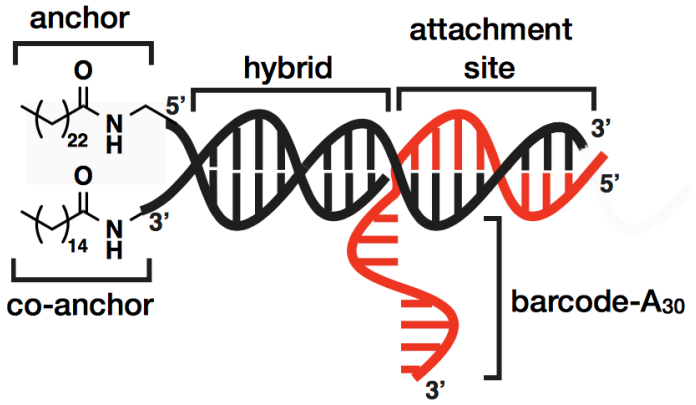

McGinnis et al. 2019

# Antibody hashing of human and mouse samples



# Lipid-modified oligo hashing

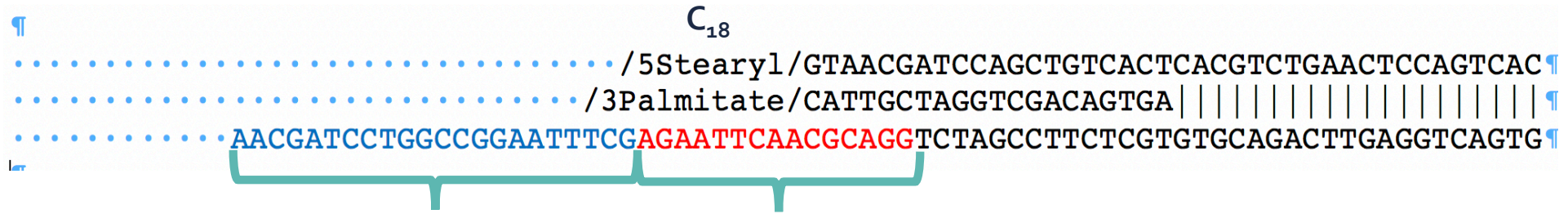
1. MULTI-seq LMO for cells



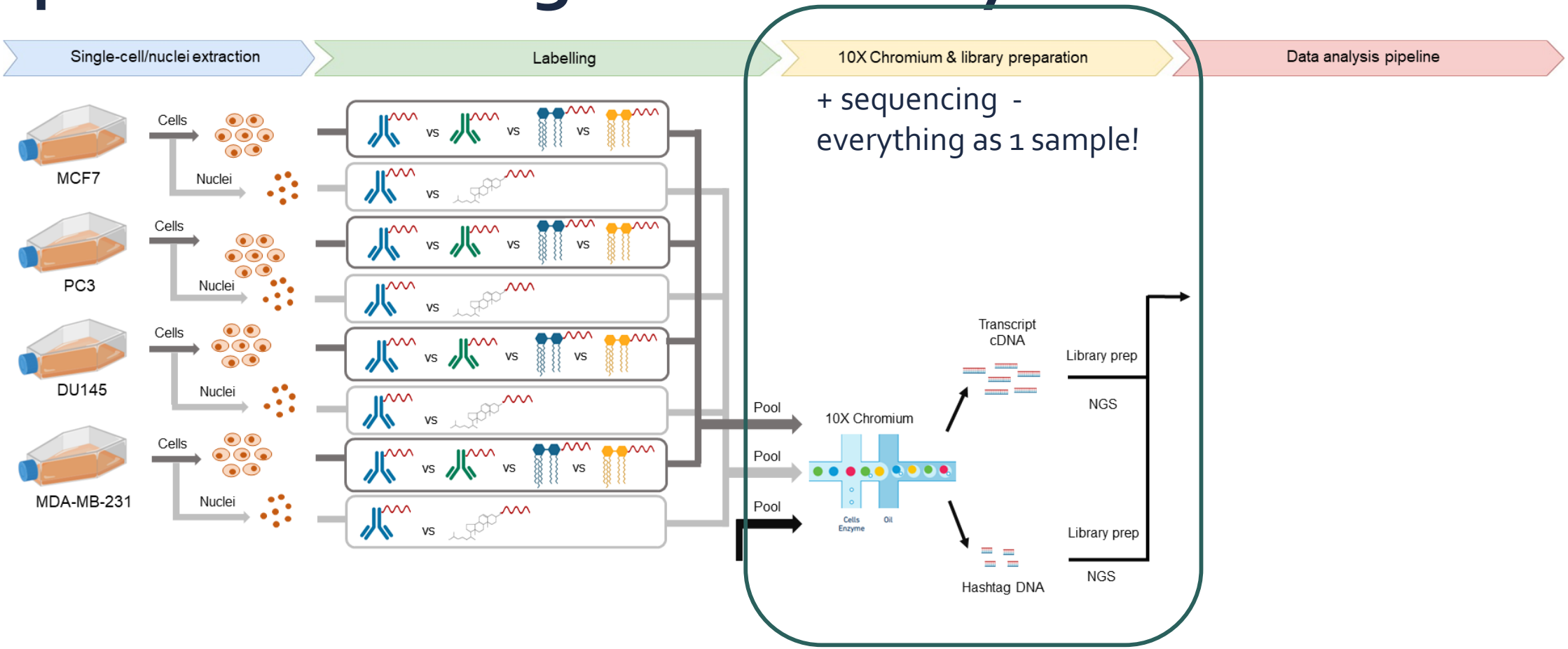

(McGinnis et al; Nature Methods; 2019)

2. CMO for nuclei

3. Custom LMO (on basis of 10x Feature Barcoding=TotalSeq B sequences)



# Experimental design of the study



# Analysis pipeline of the study

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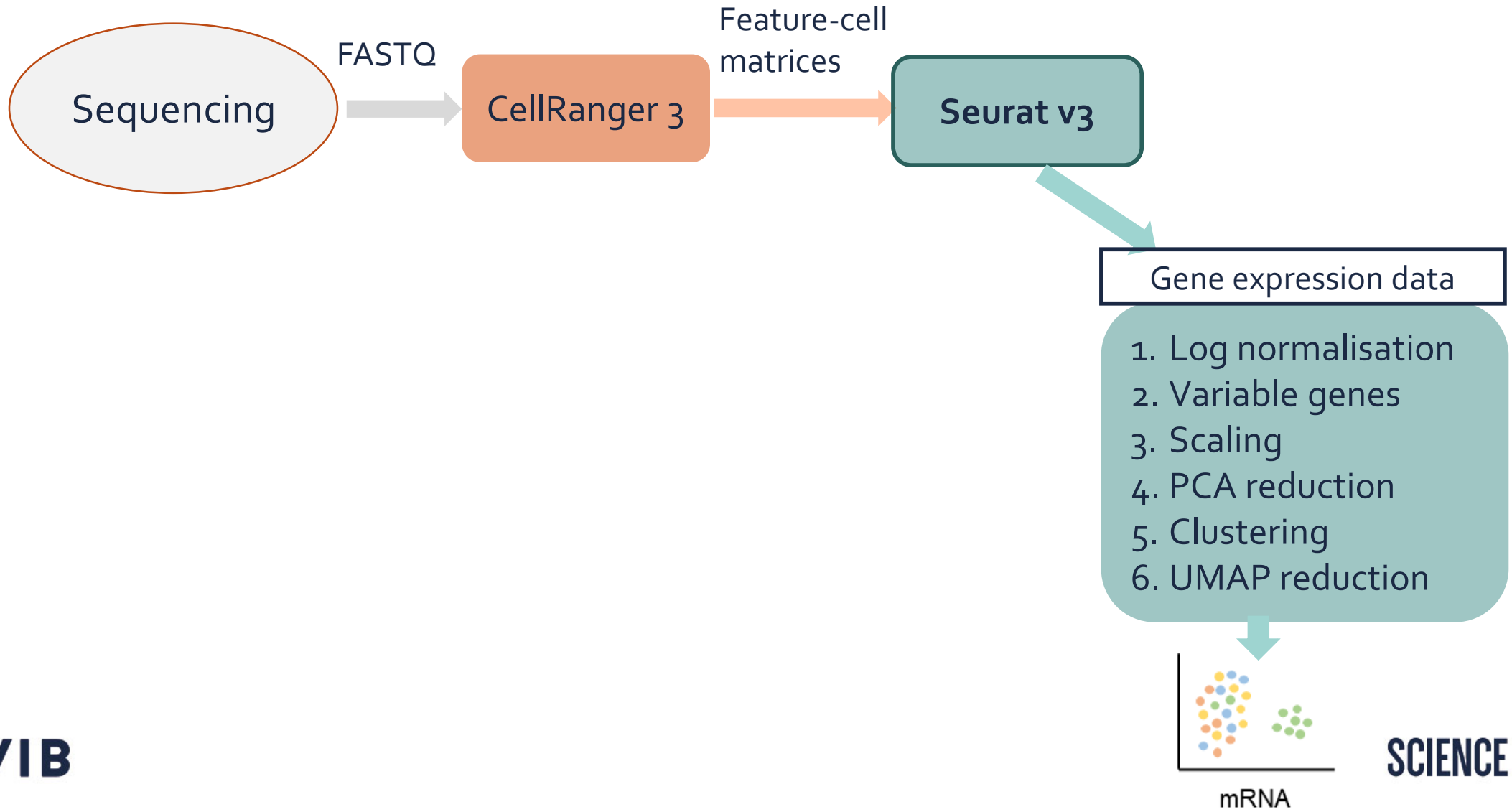
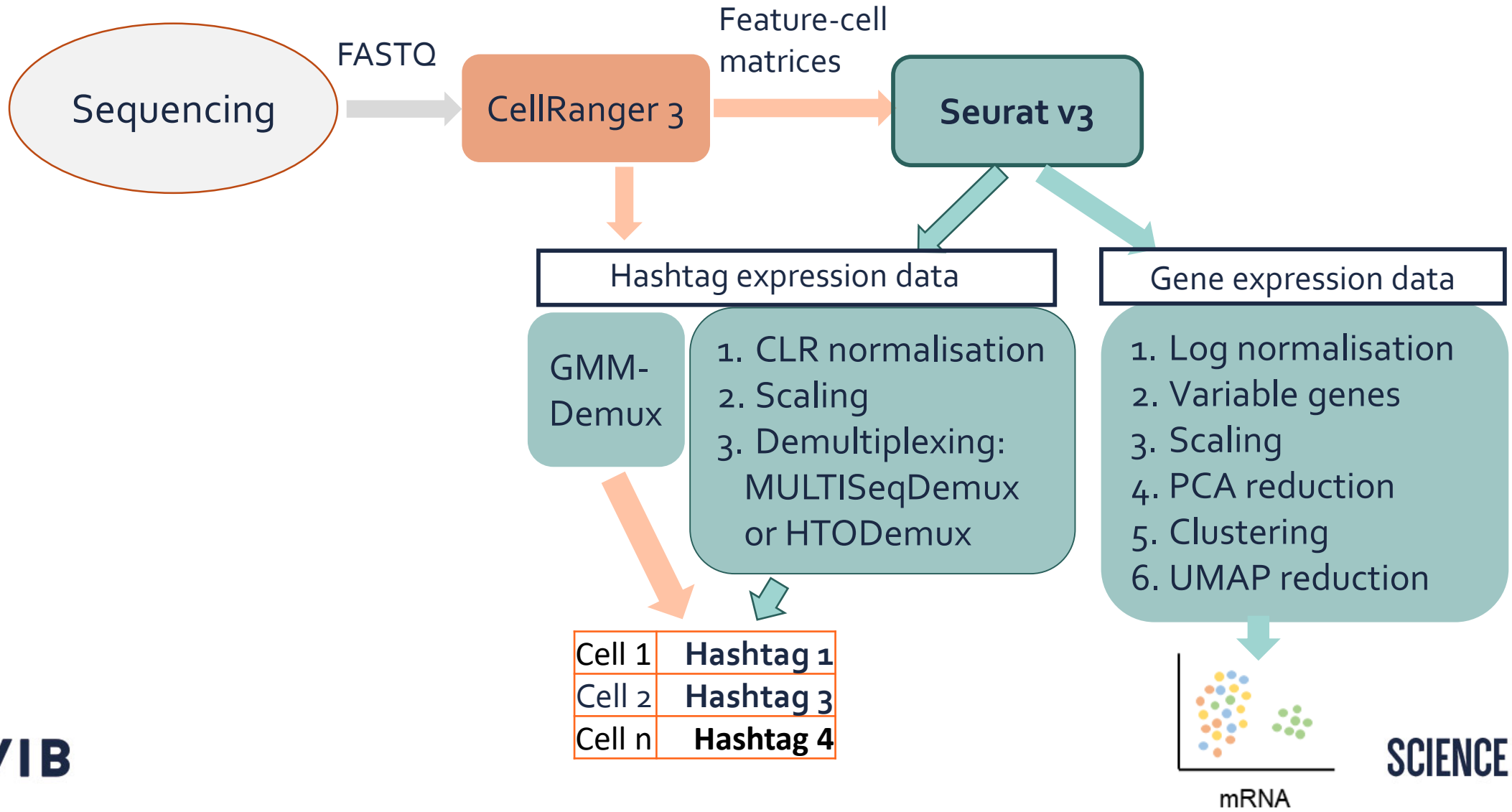


# Analysis pipeline of the study

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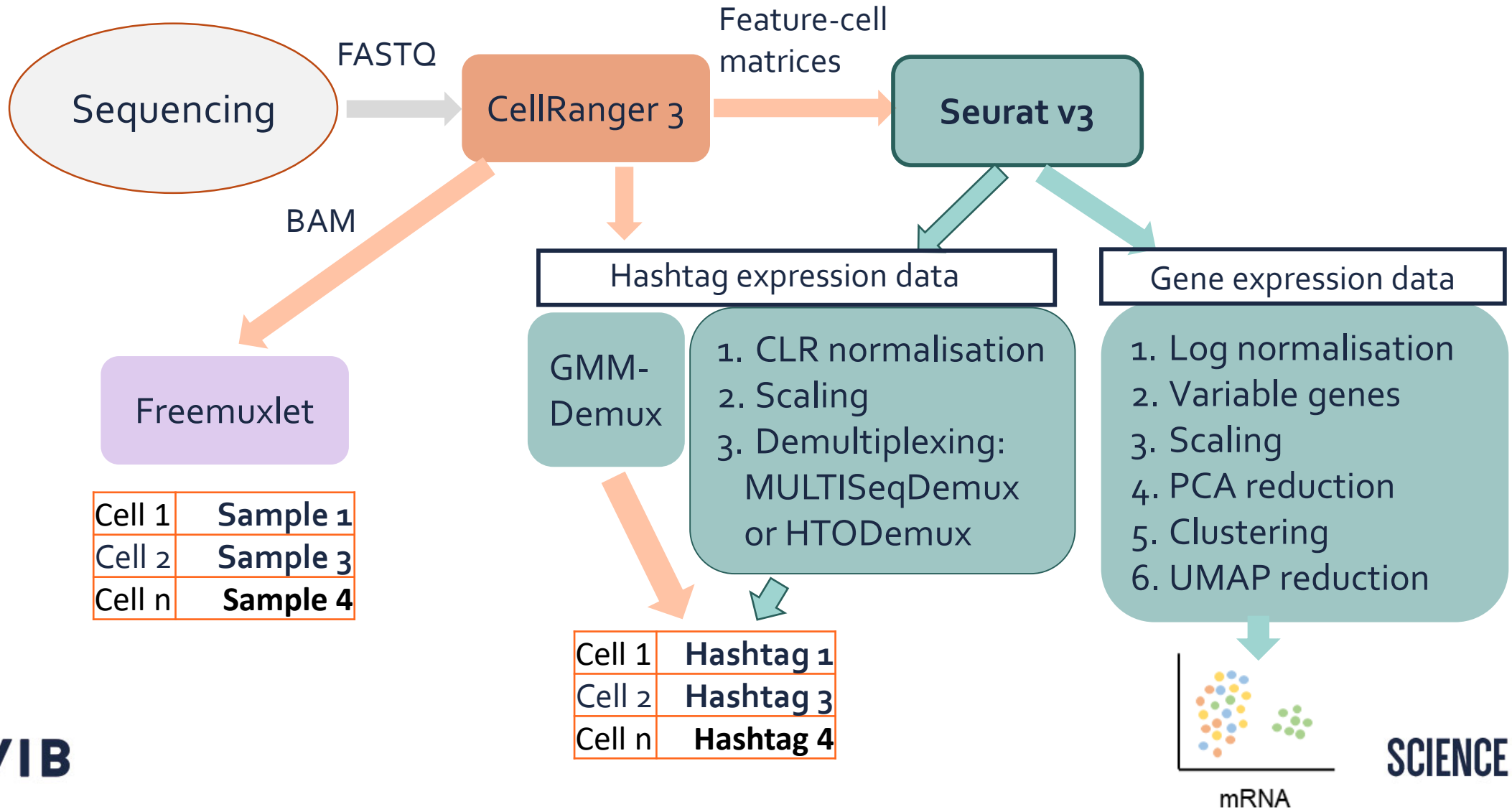
# Analysis pipeline of the study

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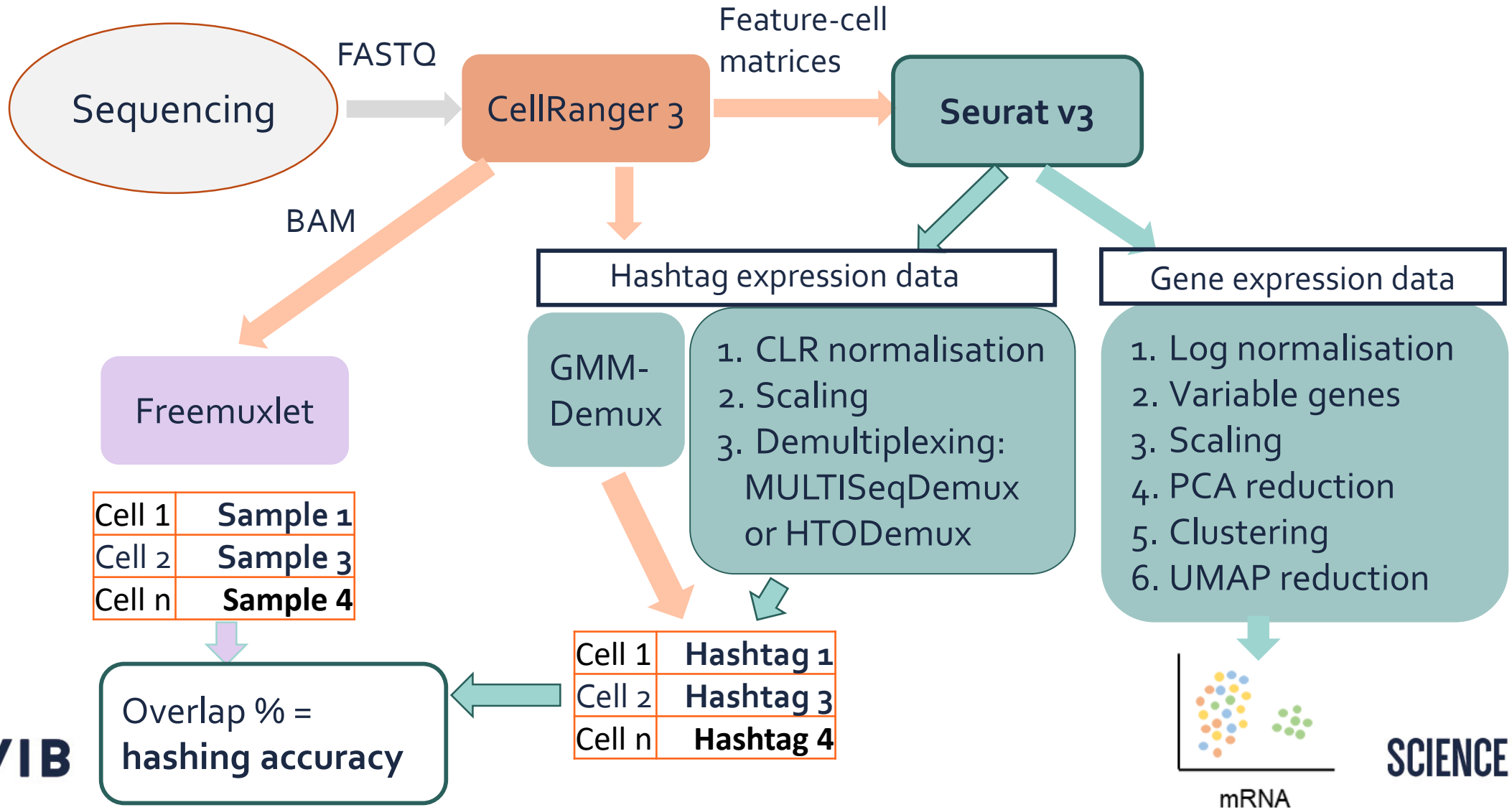


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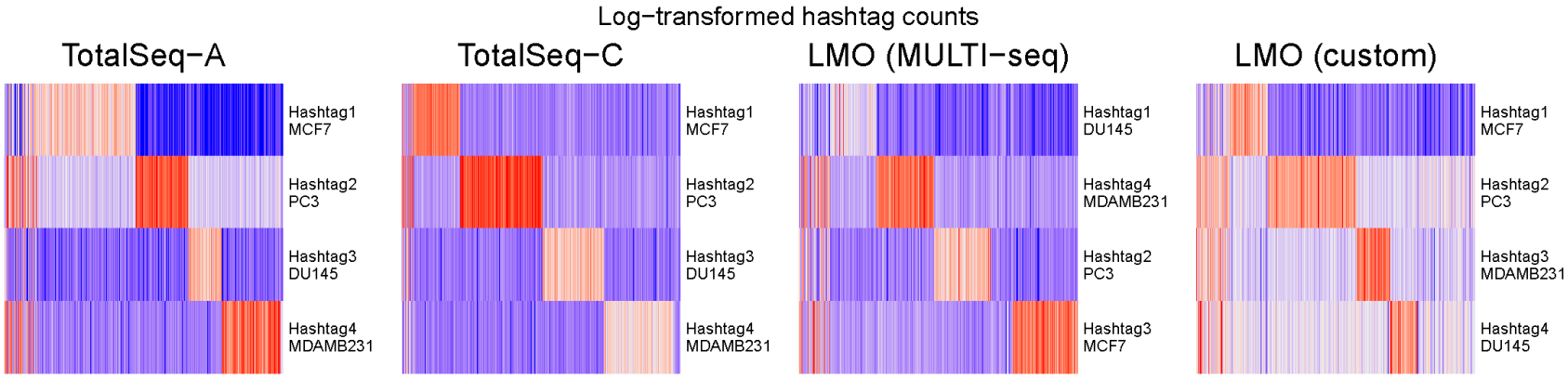
# Antibody vs lipid cell hashing



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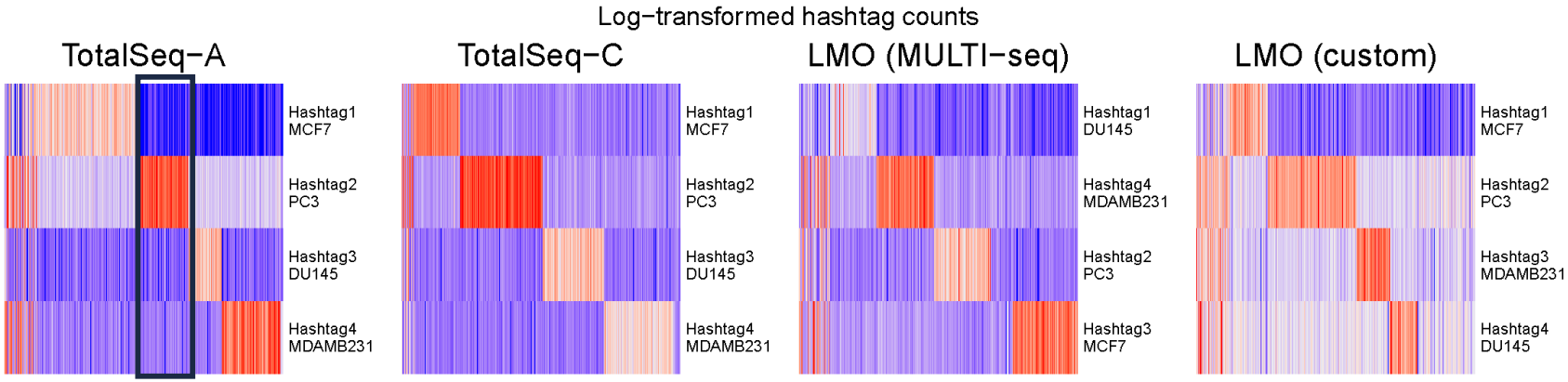
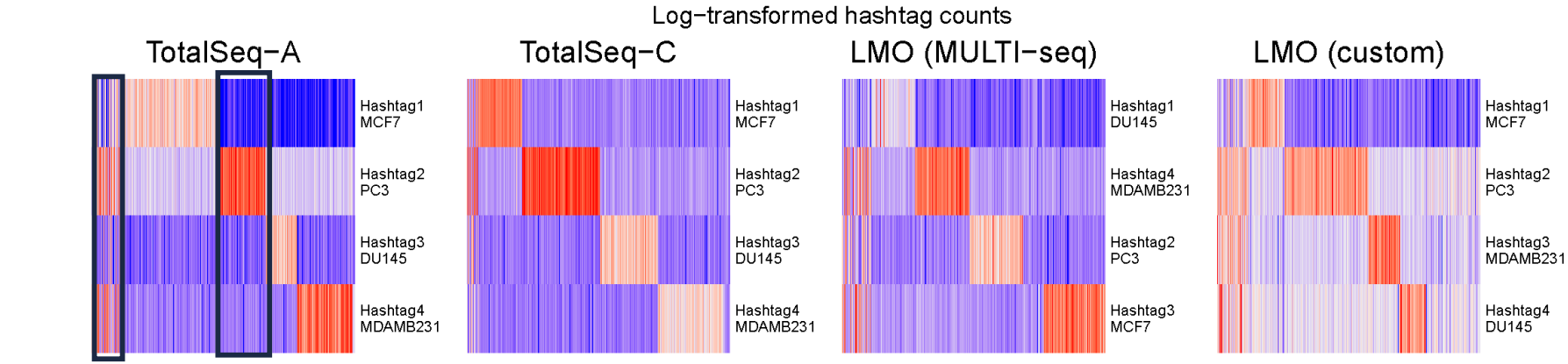


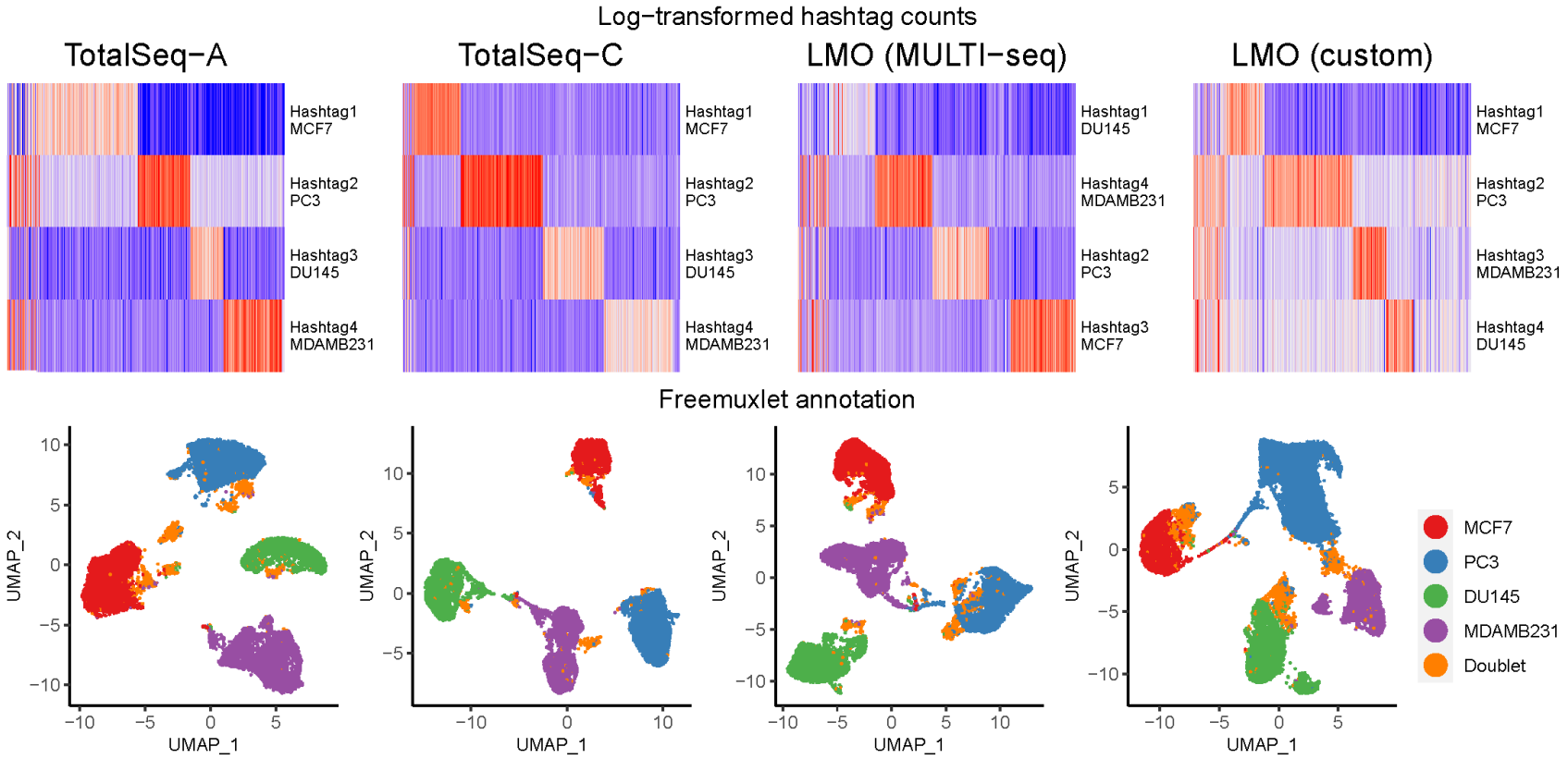

$PC_3$ cells

# Antibody vs lipid cell hashing




doublets $PC_3$ cells

# Antibody vs lipid cell hashing

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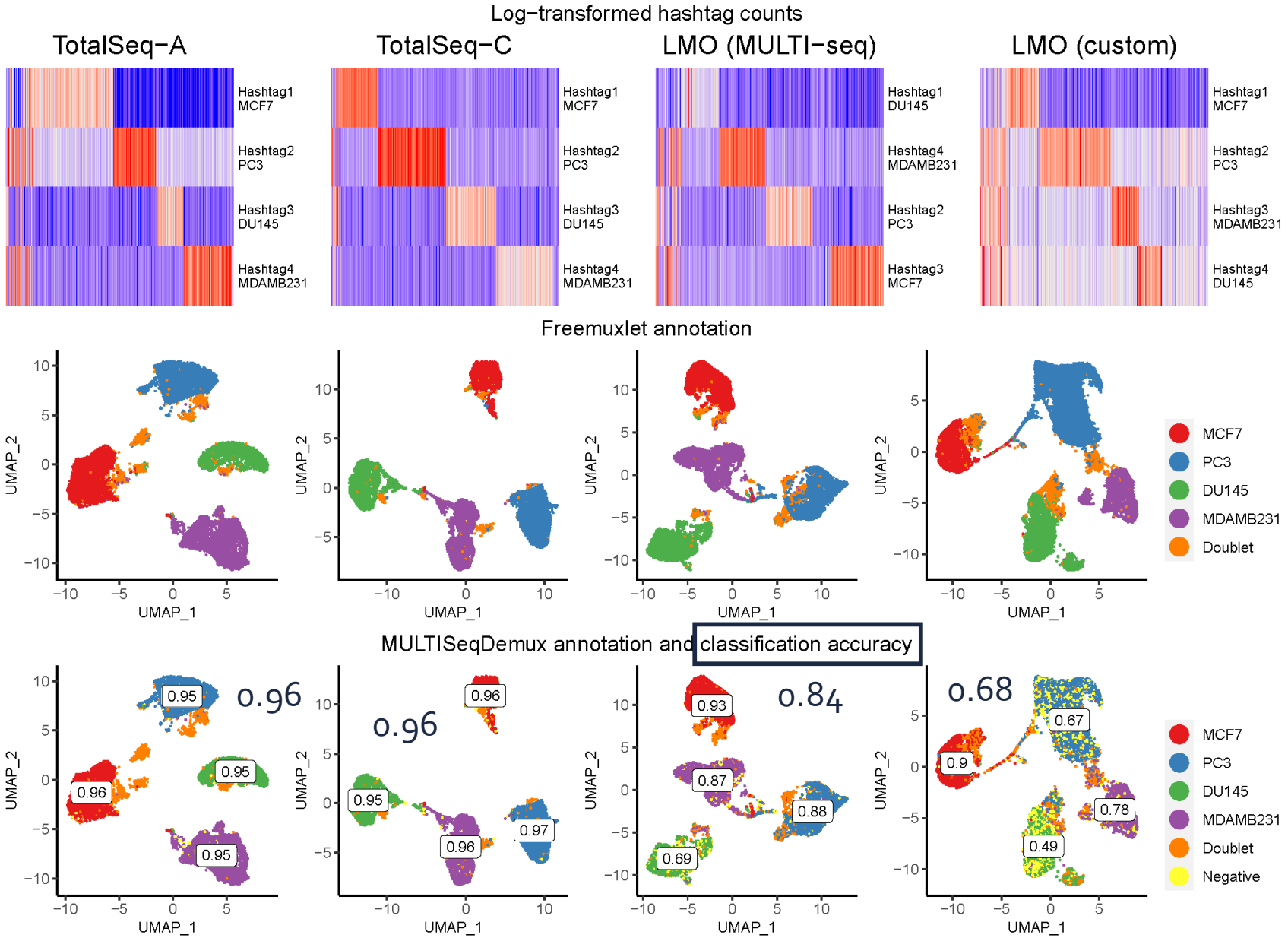


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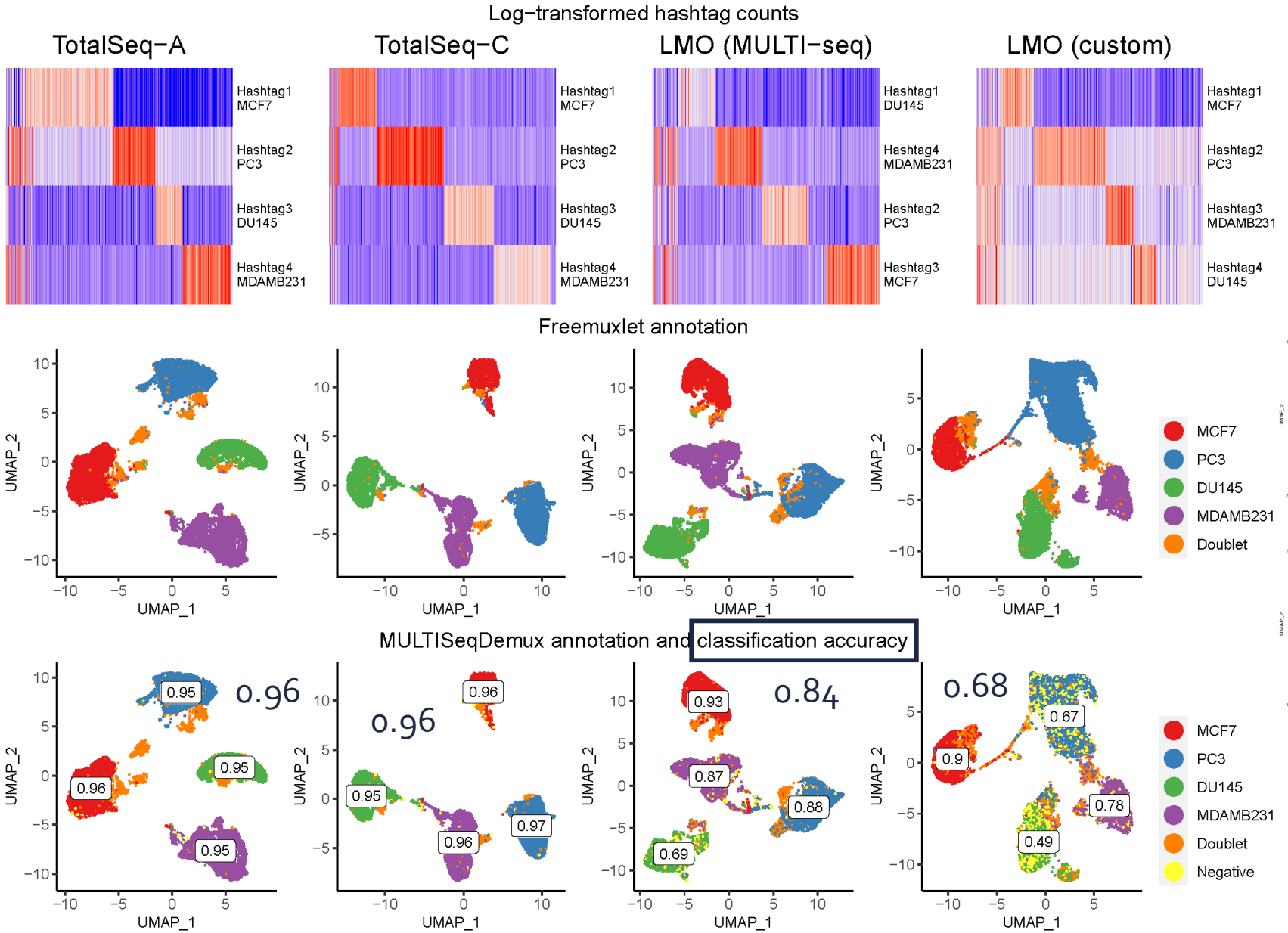


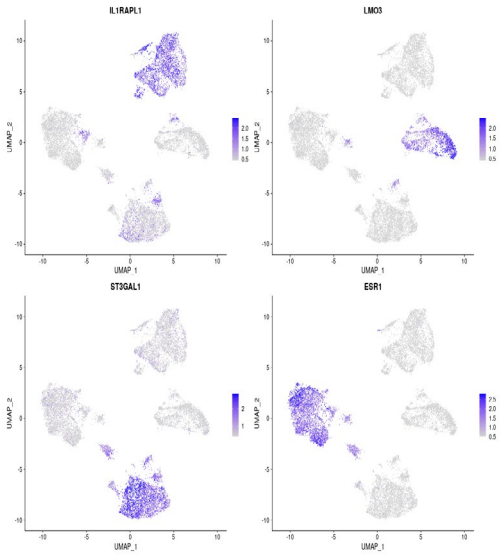


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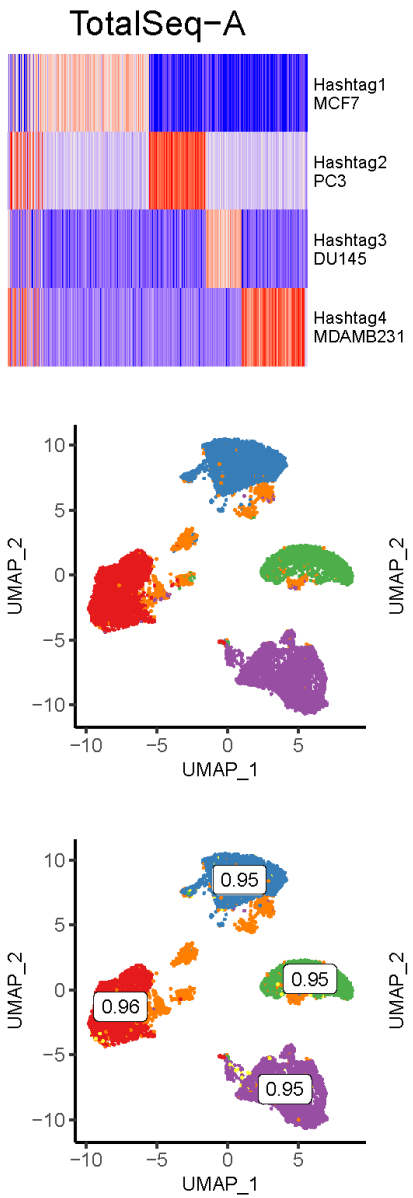
# Antibody vs lipid cell hashing




| Experiment | TotalSeq A cell | TotalSeq A cell **rep2** |
|---|---|---|
| Number of Cells (CellRanger) | 11869 | **17611** |
| Hashing accuracy (MULTISeqDemux) | 0.96 | **0.91** |

48% increase in captured cells, but 125% more doublets and 325% increase in negatives

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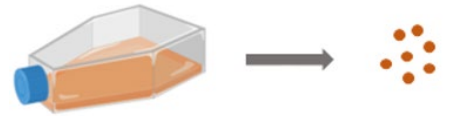
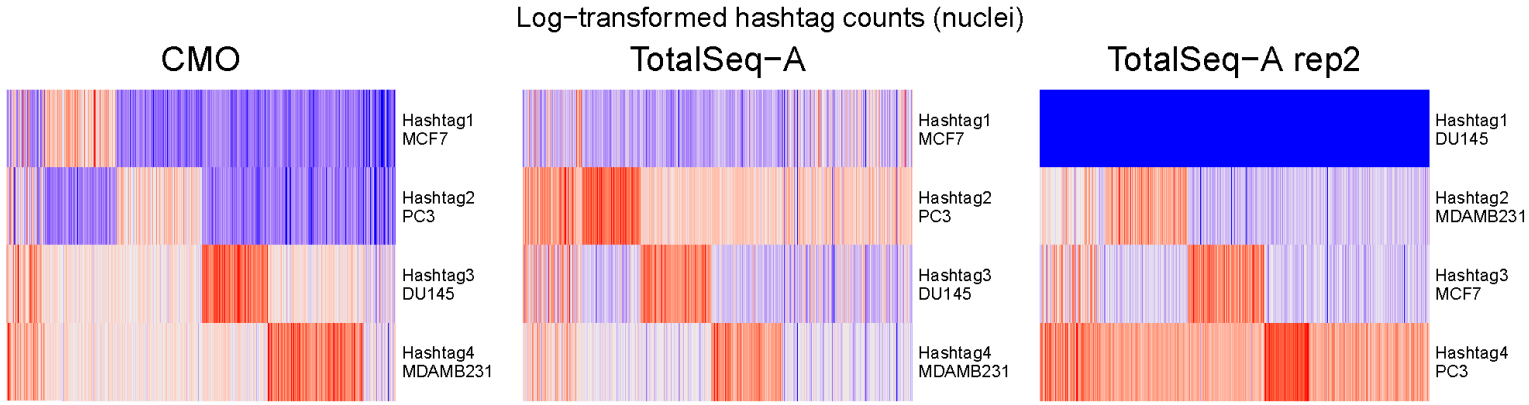
# Cholesterol vs antibody nuclei hashing





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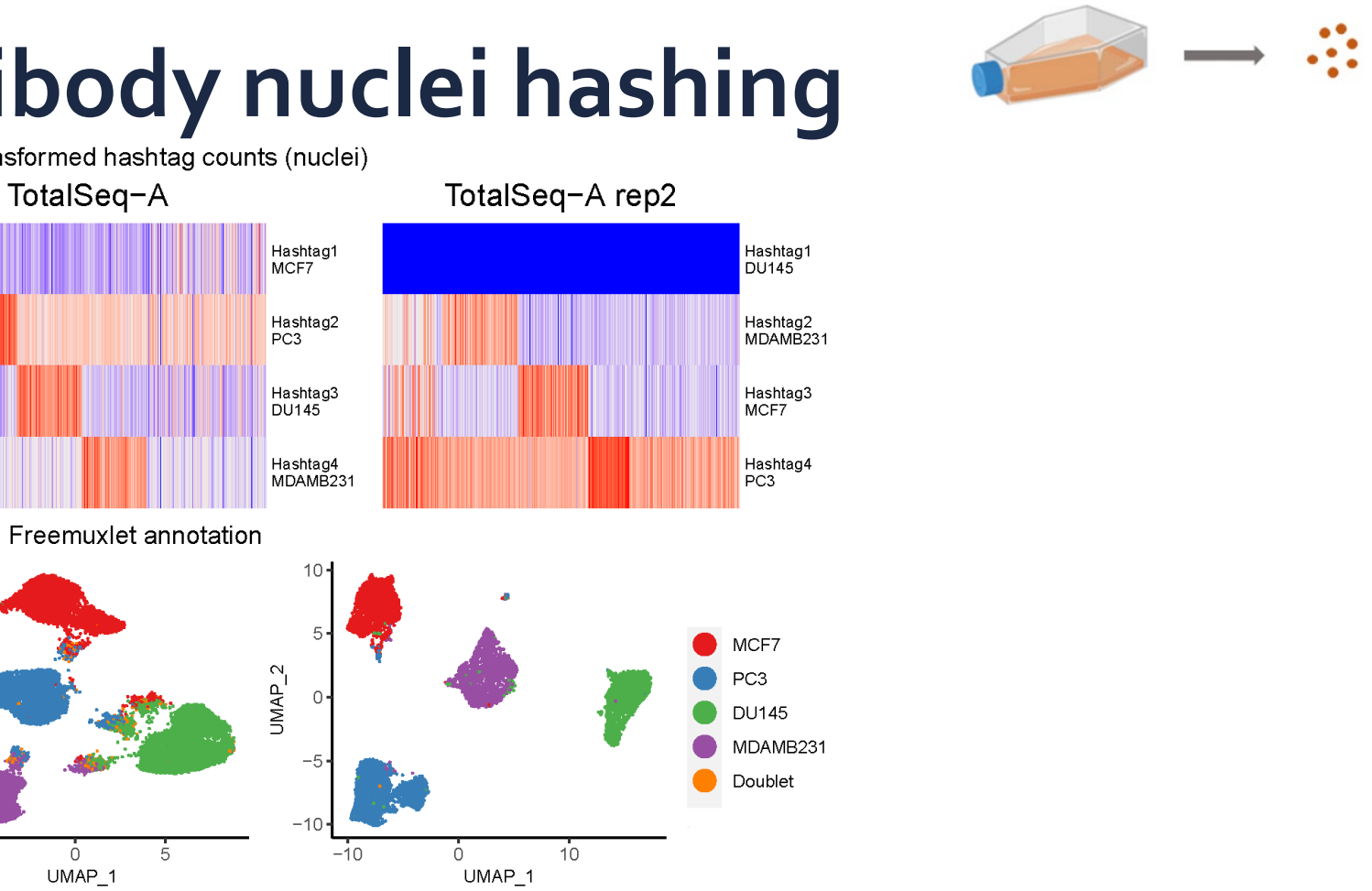

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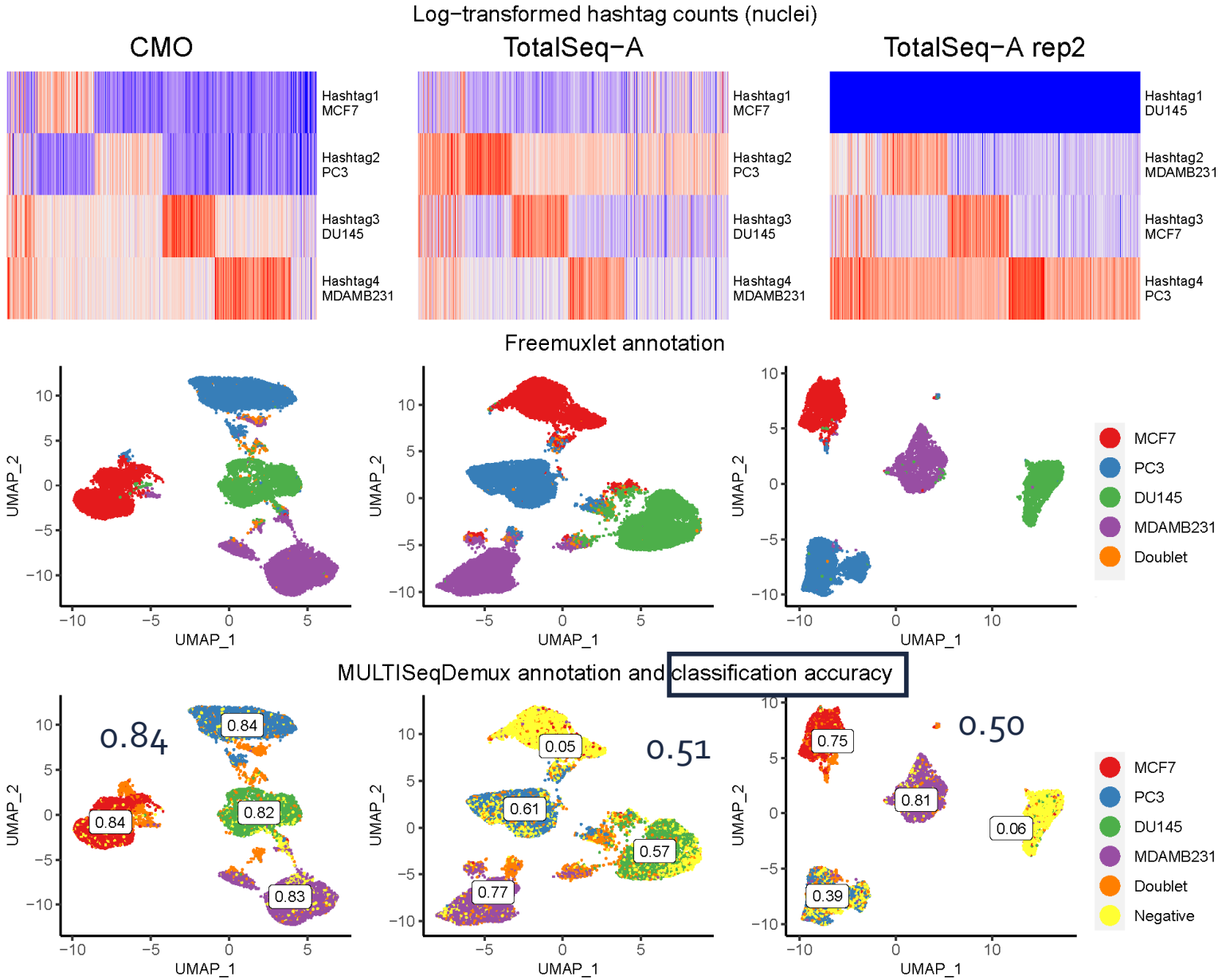


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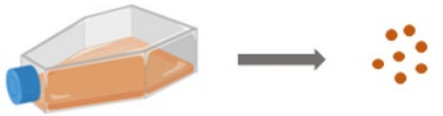
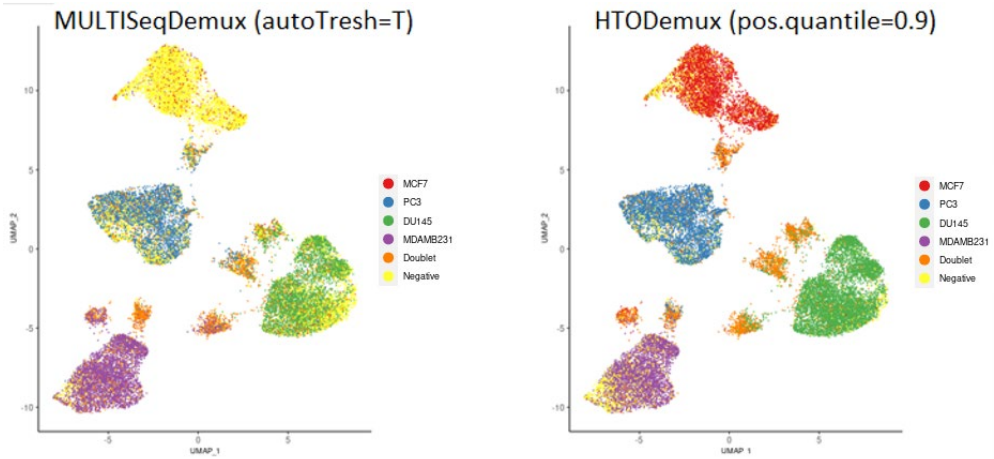
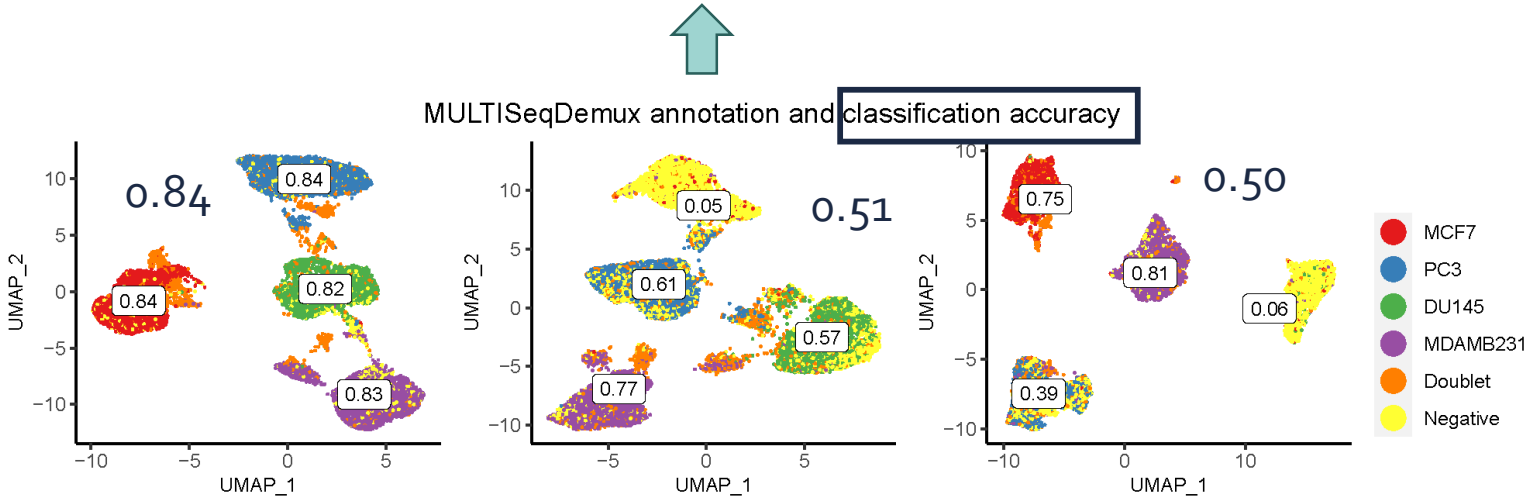


**Finetuned demultiplexing**




**Finetuned demultiplexing**

| Experiment | TotalSeq A nuclei | TotalSeq A nuclei |
|---|---|---|
| Number of Cells (CellRanger) | **23451** | **23451** |
| Hashing accuracy (MULTISeqDemux) | **0.51** | **0.65** |

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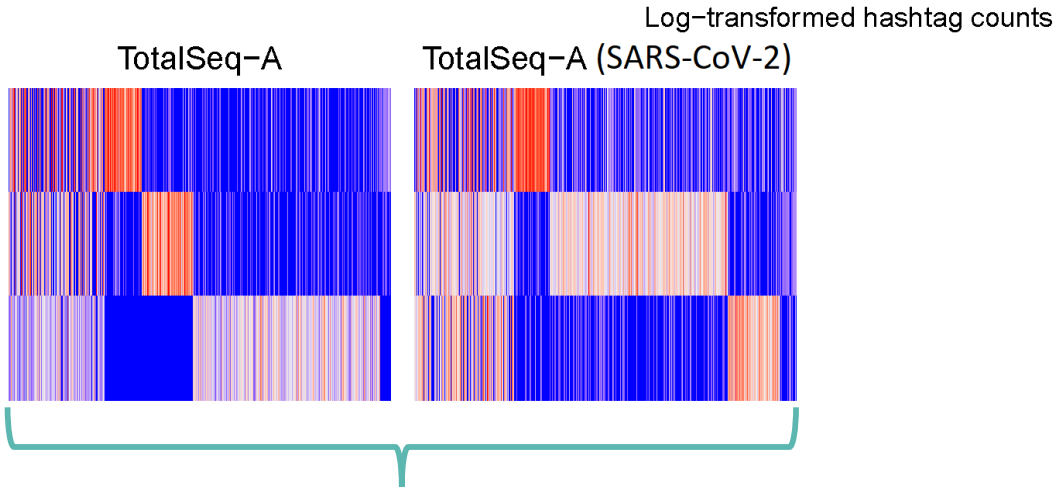
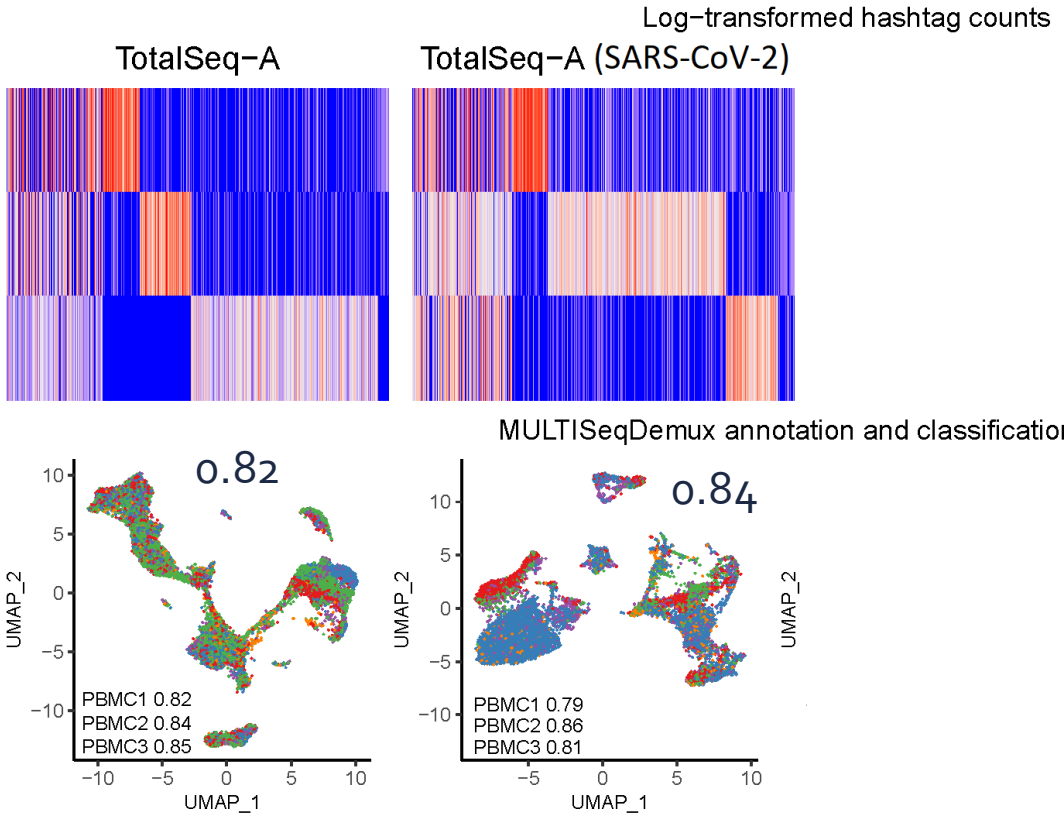
# Human PBMC hashing




Additional CITE-seq panel of 277 TotalSeq A antibodies

# Human PBMC hashing

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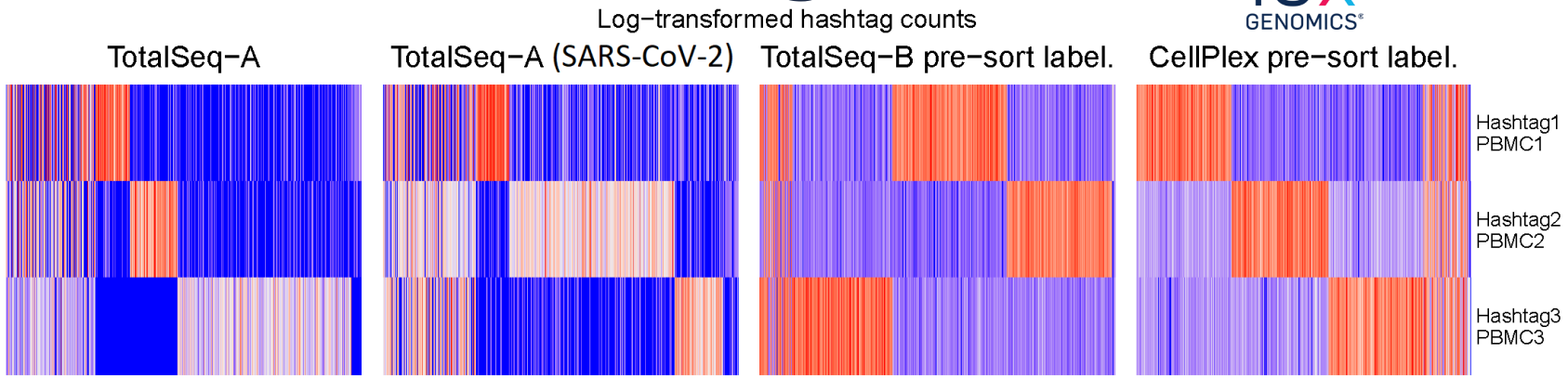


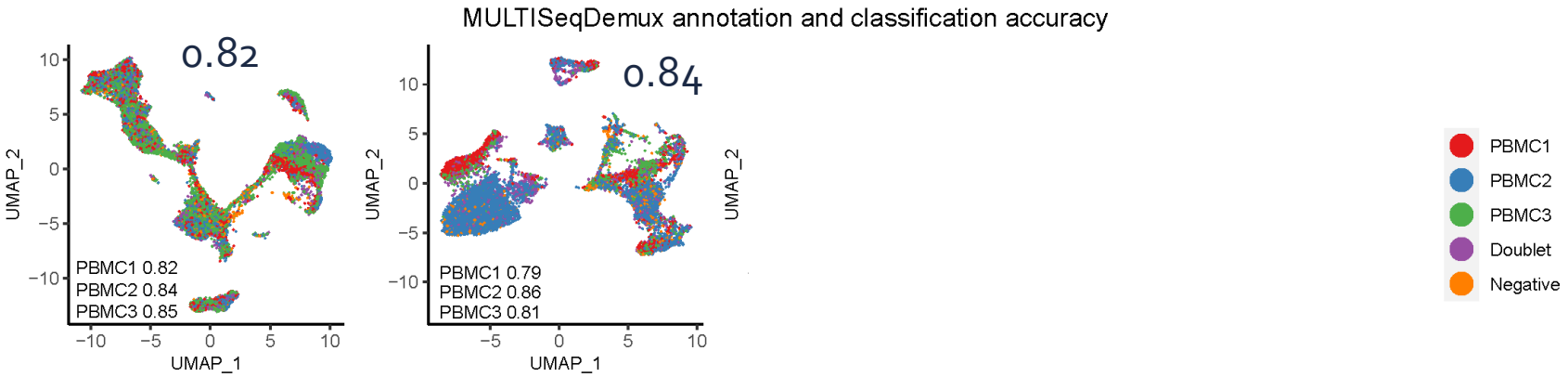


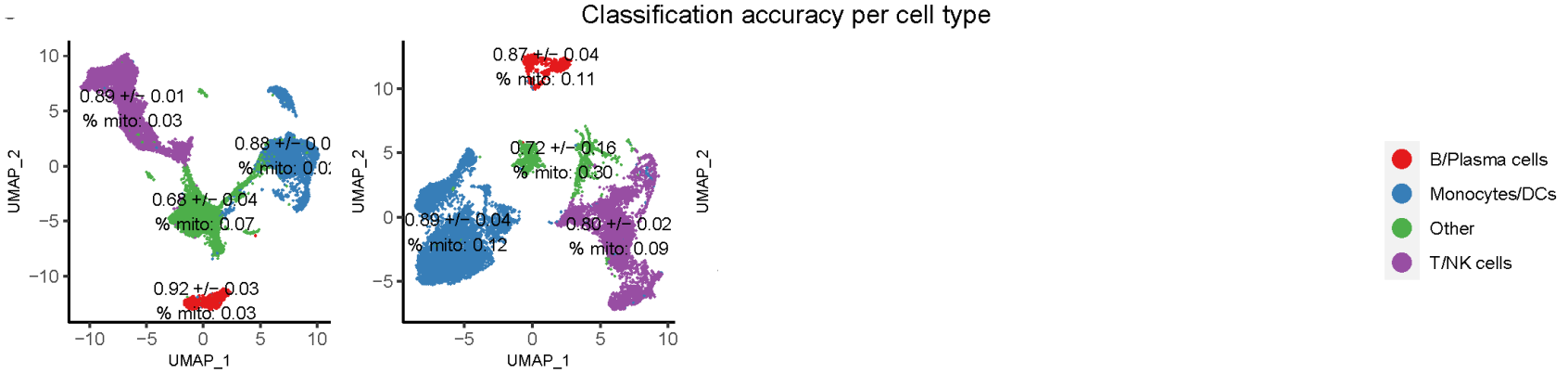


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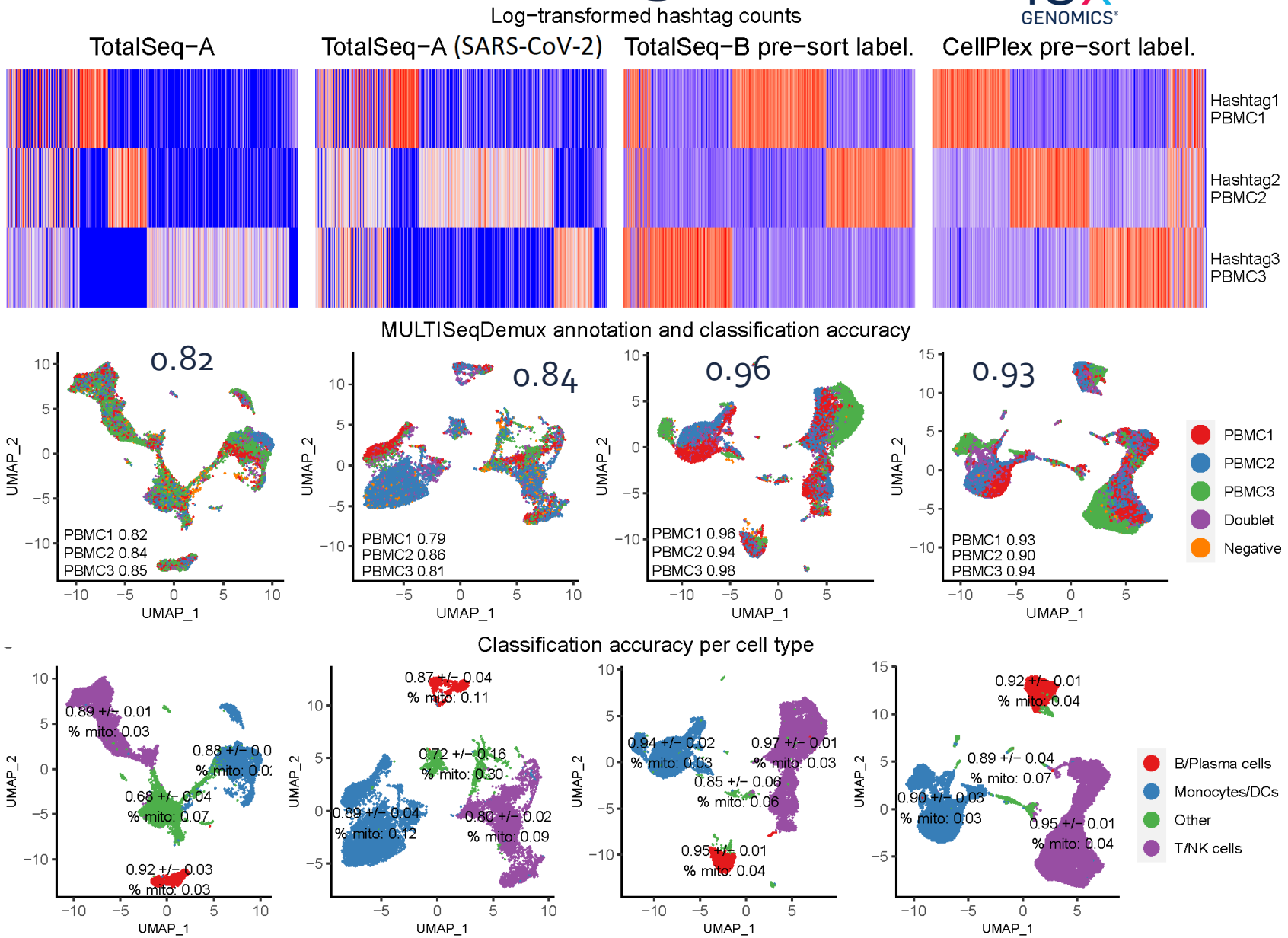


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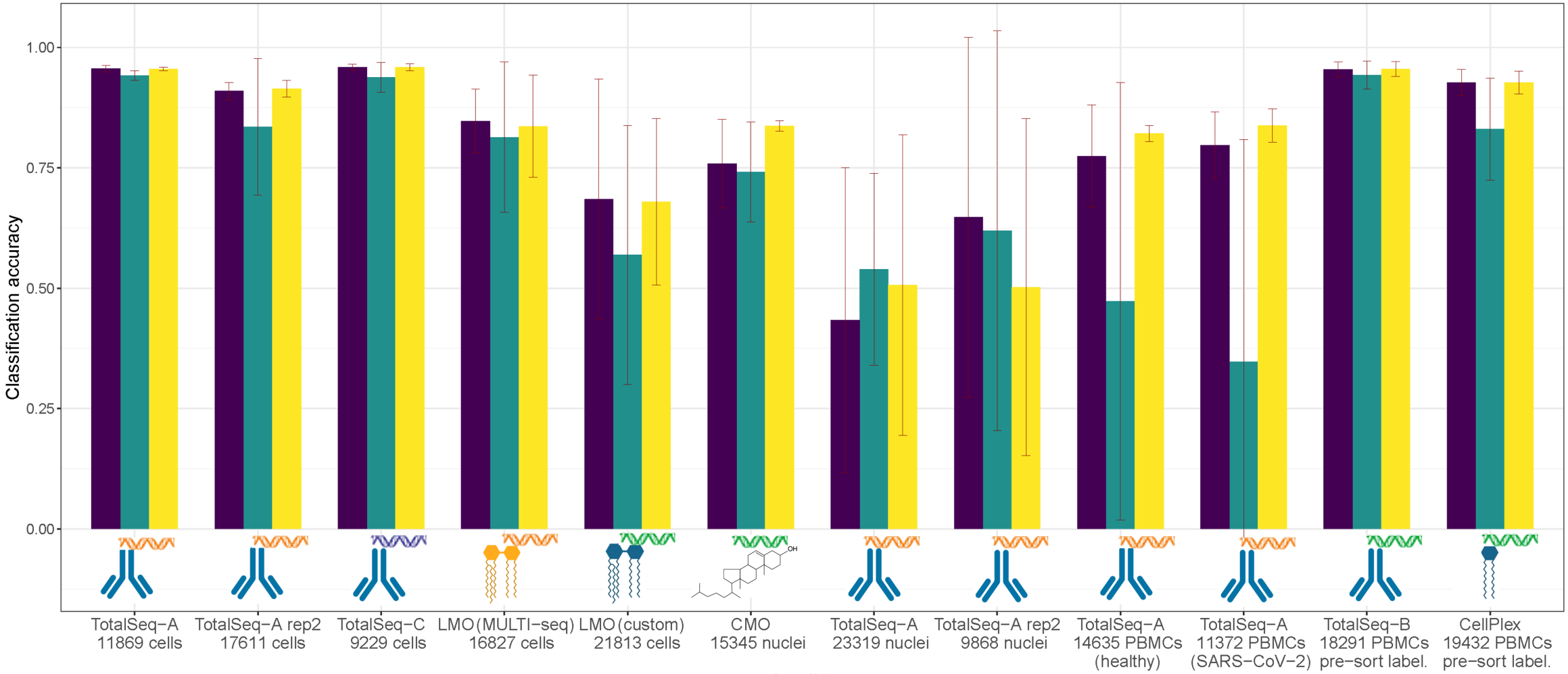
# Overview of human cell and nuclei hashing




Conditions

4x 1390 HTO reads per cell

4x 860 HTO reads per nuclei

3x 1210 HTO reads per cell

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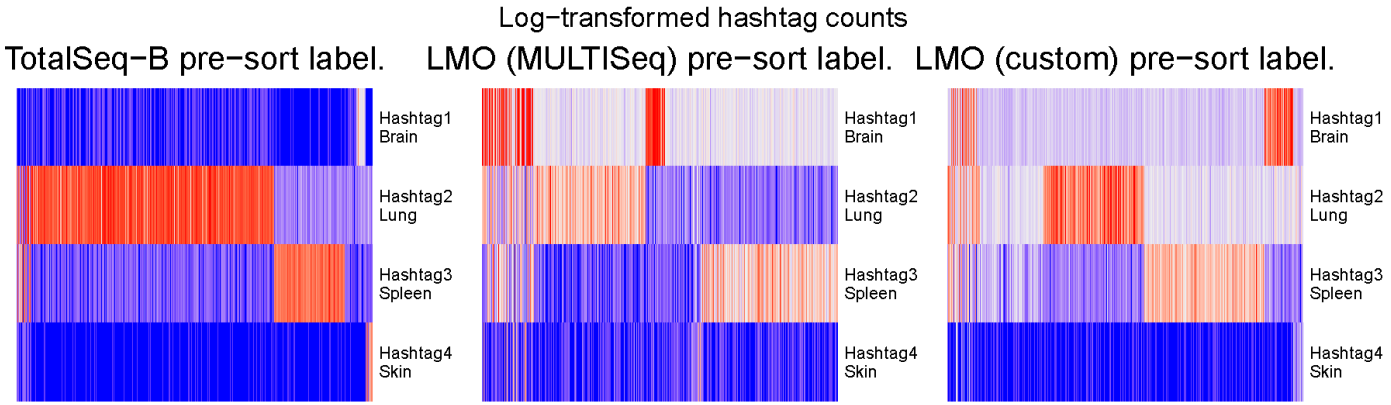
# Mice tissue hashing

BALB/c

C57BL6J

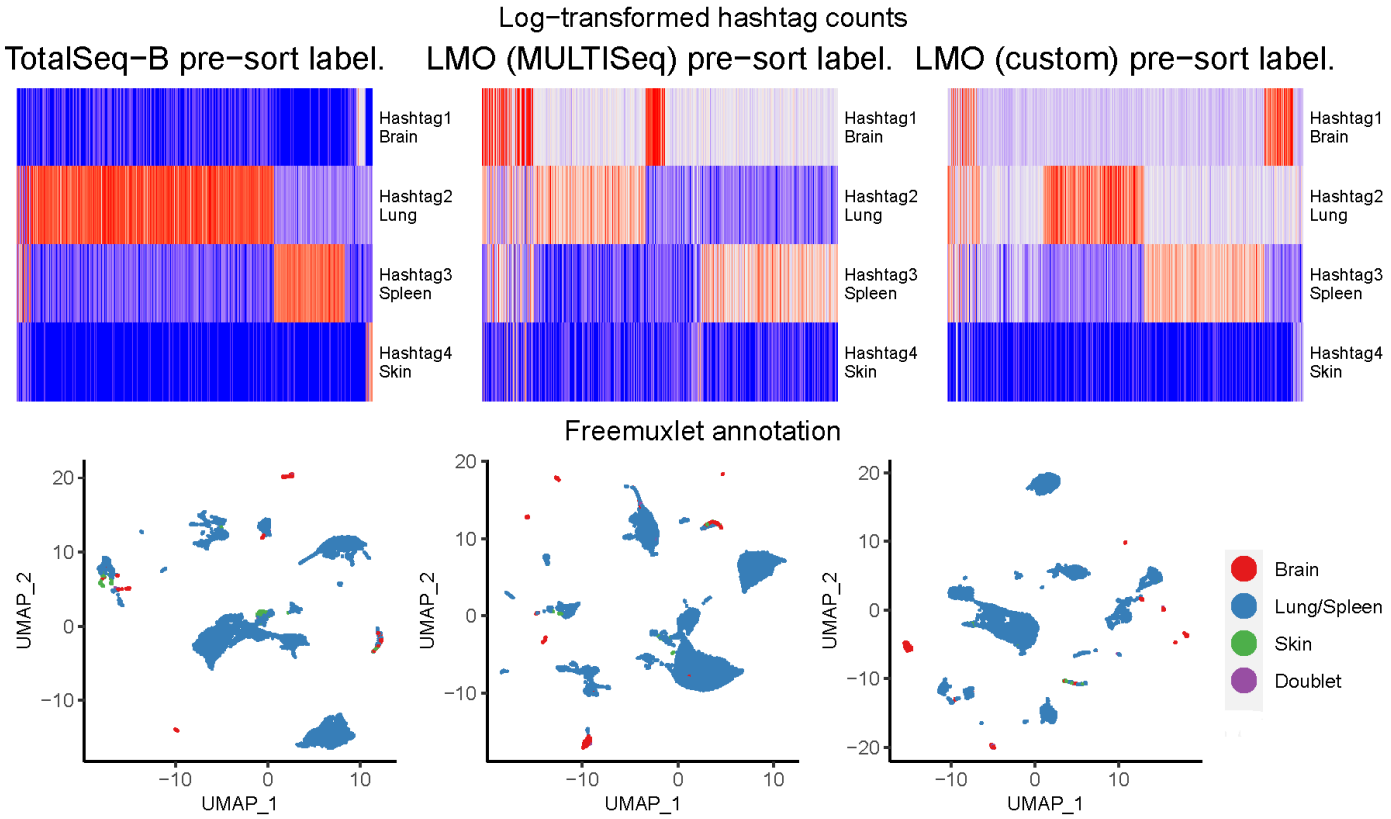
C57BL6N

129SVEvs7



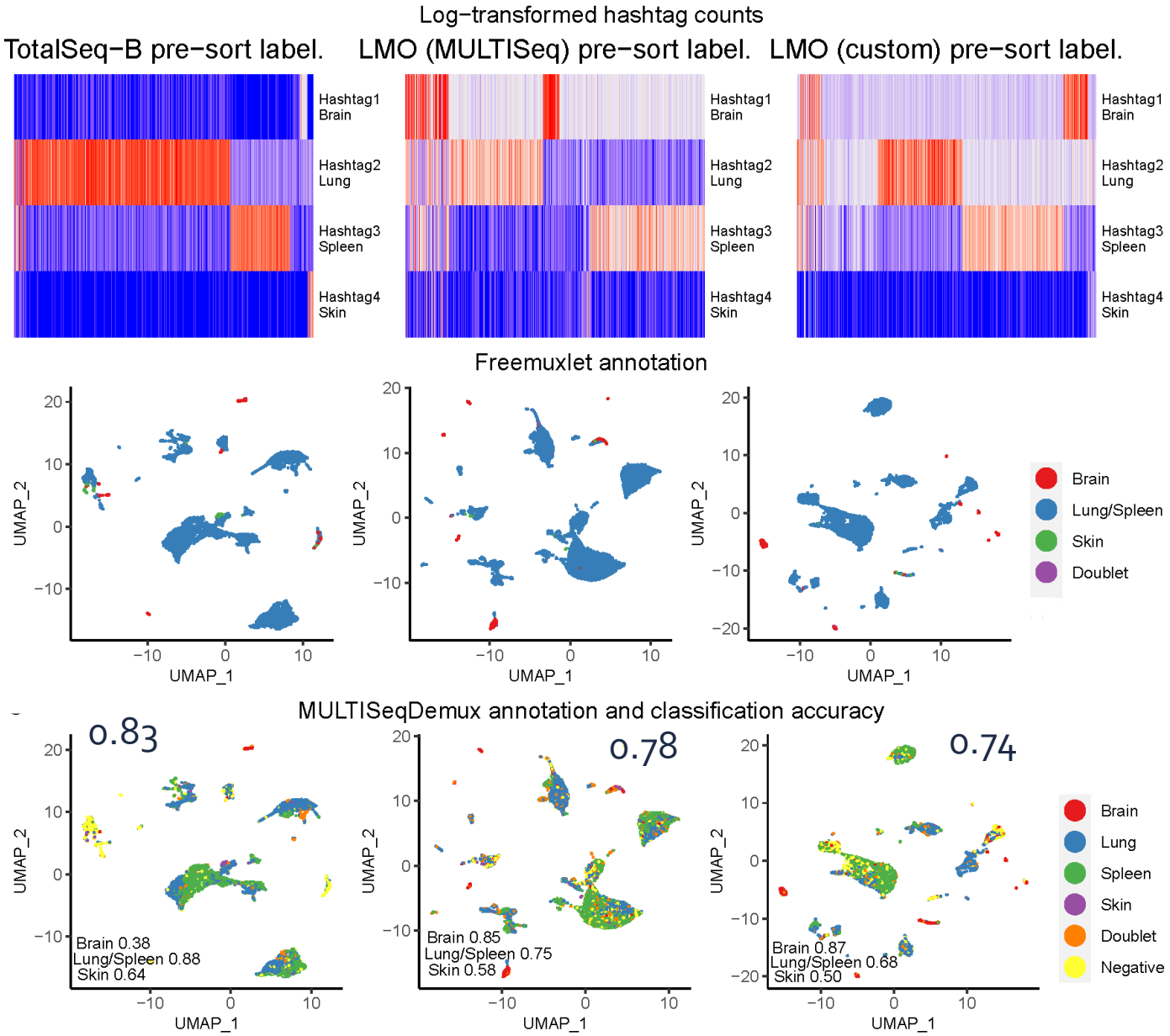
# Mice tissue hashing

- BALB/c
- C57BL6J
- C57BL6N
- 129SVEvs7



# Mice tissue hashing

- BALB/c
- C57BL6J
- C57BL6N
- 129SVEvs7

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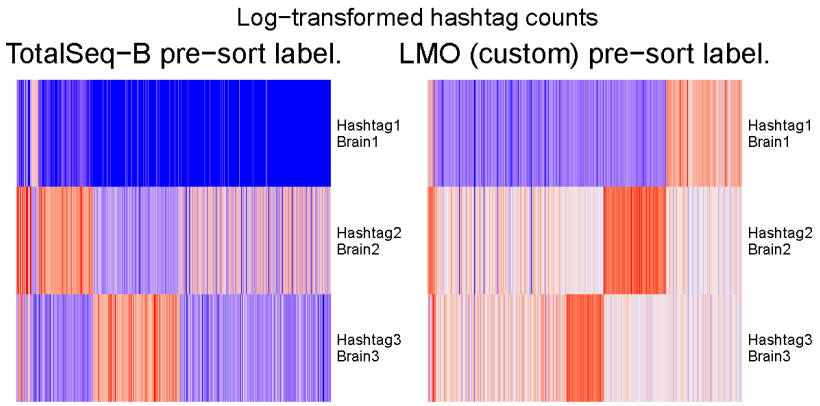
# Mice brain cell hashing

BALB/c

C57BL6N

DBA2J

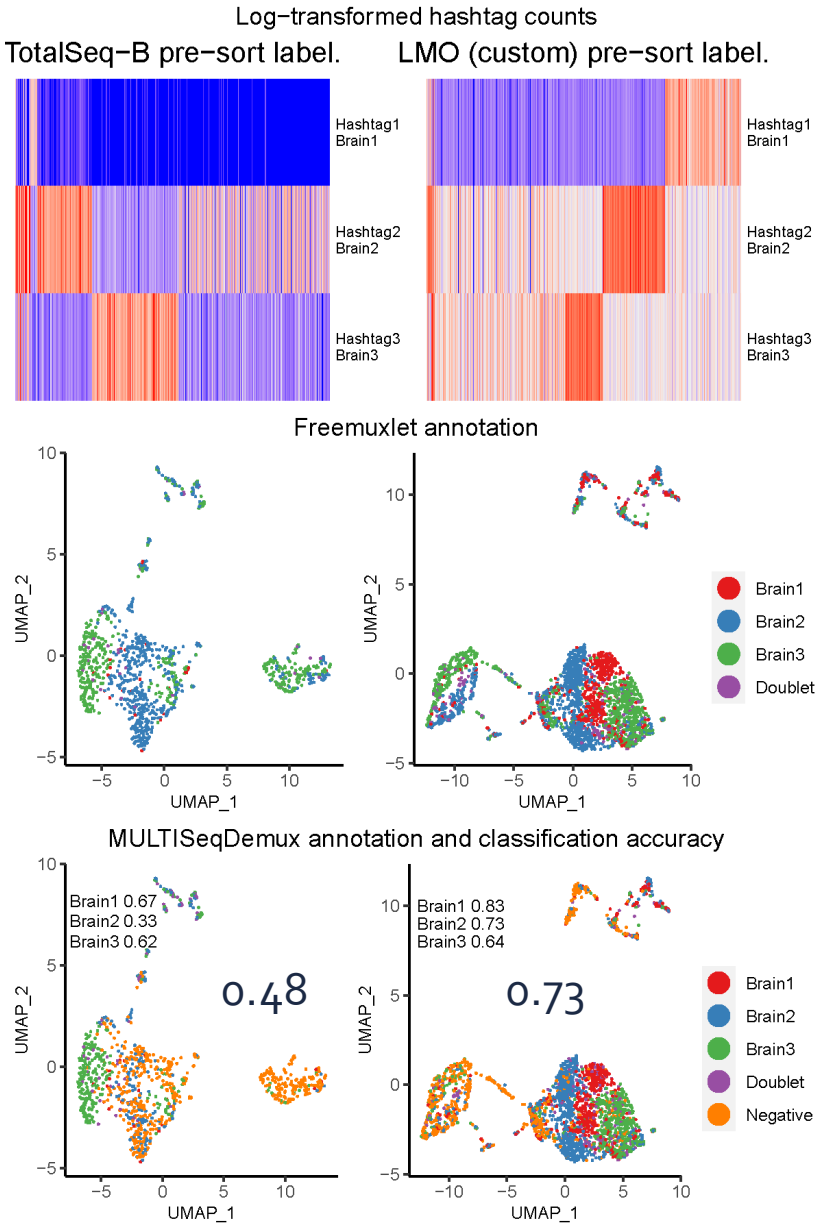


# Mice brain cell hashing


BALB/c


C57BL6N


DBA2J

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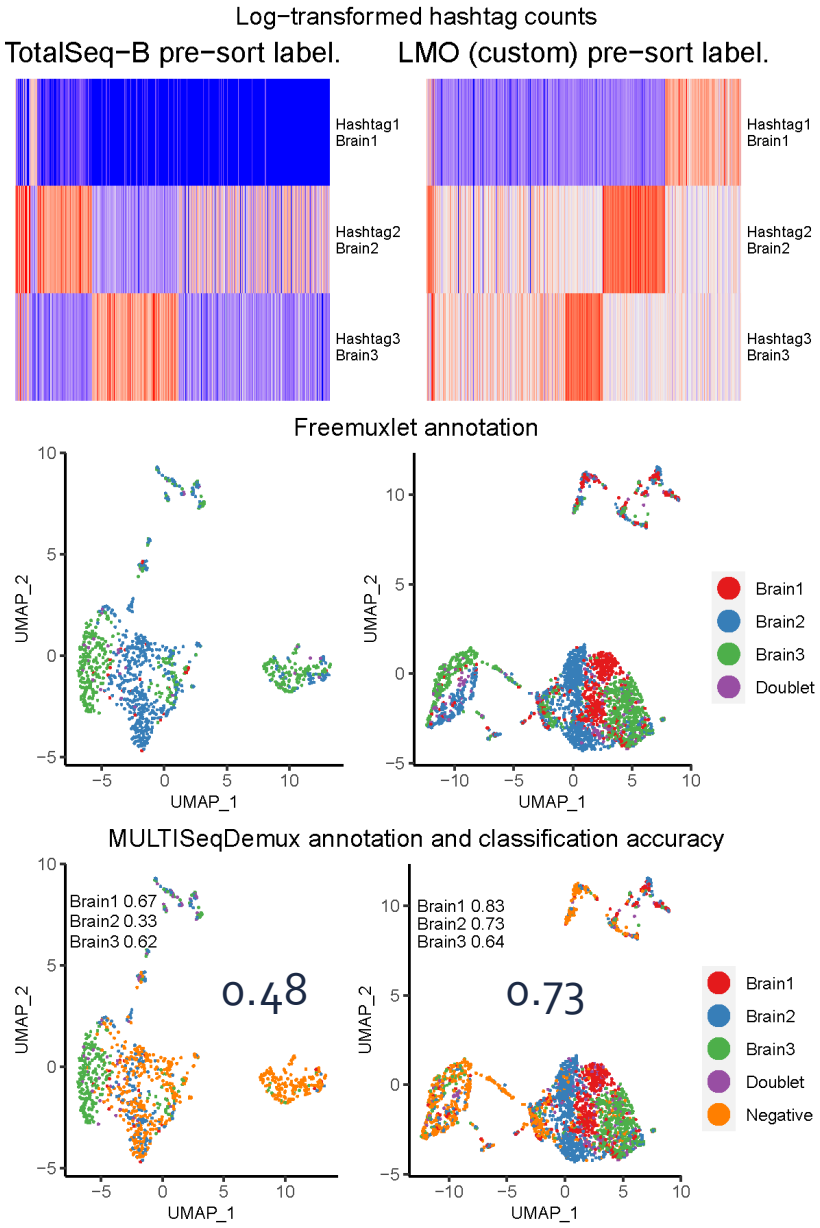
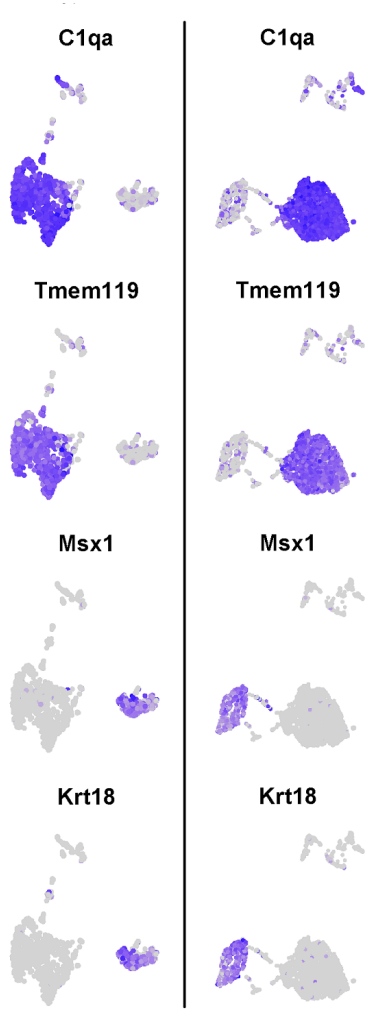
# Mice brain cell hashing


BALB/c


C57BL6N


DBA2J







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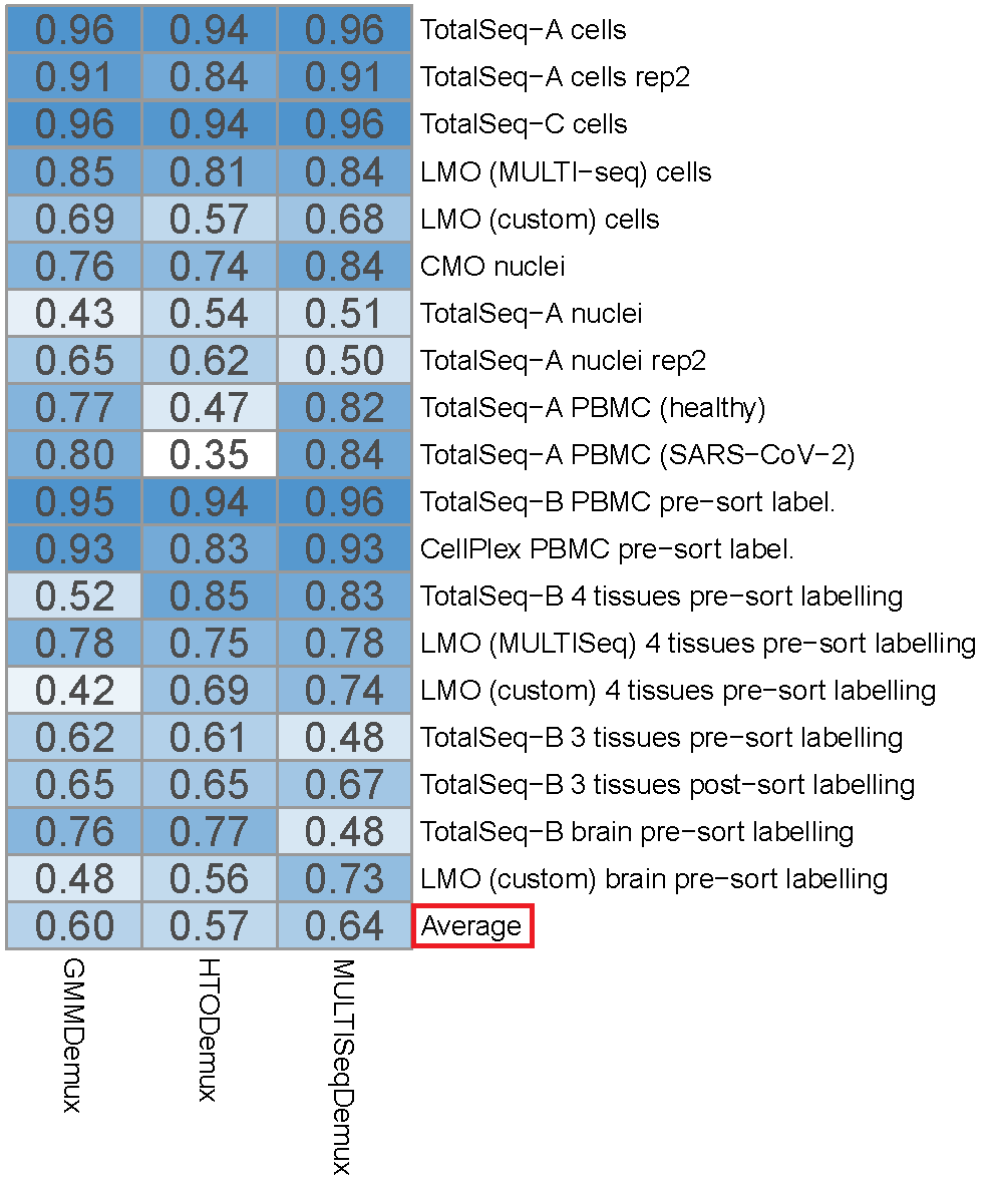
# Comparison of demultiplexing functions




Mylka et al., Genome Biology, 2022

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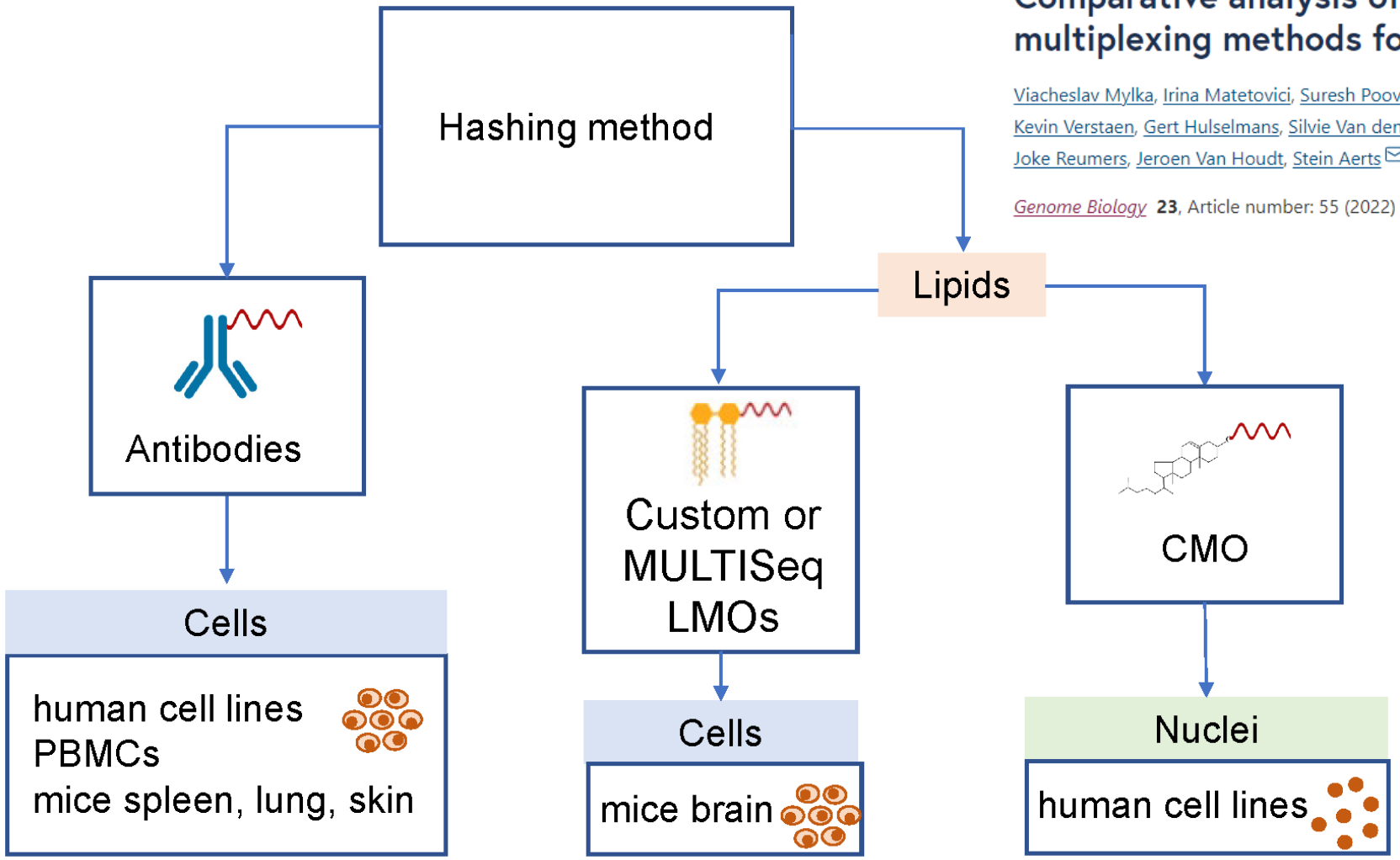
# Hashing strategies: which one to use?

Research | Open Access | Published: 16 February 2022

## Comparative analysis of antibody- and lipid-based multiplexing methods for single-cell RNA-seq

Viacheslav Mylka, Irina Matetovici, Suresh Poovathingal, Jeroen Aerts, Niels Vandamme, Ruth Seurinck, Kevin Verstaen, Gert Hulselmans, Silvie Van den Hoecke, Isabelle Scheyltjens, Kiavash Movahedi, Hans Wils, Joke Reumers, Jeroen Van Houdt, Stein Aerts & Yvan Saeys

*Genome Biology* **23**, Article number: 55 (2022) | Cite this article

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# Conclusions

1. Antibody-based hashing is the most efficient protocol on human cell lines, PBMCs, mice lung and spleen.
2. Lipid hashing delivers the best results on immune mice brain cells and nuclei from human cell lines
3. Hashing can be applied on PBMCs from healthy individuals and SARS-CoV-2 patients
4. MULTISeqDemux (autoTresh=T) is the preferred demultiplexing function

# Acknowledgments


VIB-UGENT CENTER FOR INFLAMMATION RESEARCH


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Kevin Verstaen


Kia Movahedi


Isabelle Scheyltjens

VIB-KU LEUVEN CENTER FOR BRAIN & DISEASE RESEARCH


Stein Aerts


Suresh Poovathingal


Gert Hulselmans

VIB tech watch


Viacheslav Mylka


Irina Matetovici


Jeroen Aerts


Silvie Van Den Hoecke

VIB FLOW CORE


Gert Van Isterdael

VIB

janssen PHARMACEUTICAL COMPANIES OF Johnson&Johnson


Jeroen Van Houdt


Hans Wils


Joke Reumers

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# Thank you!