

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

Viacheslav Mylka

VIB-Janssen Single Cell Tech Collaboration

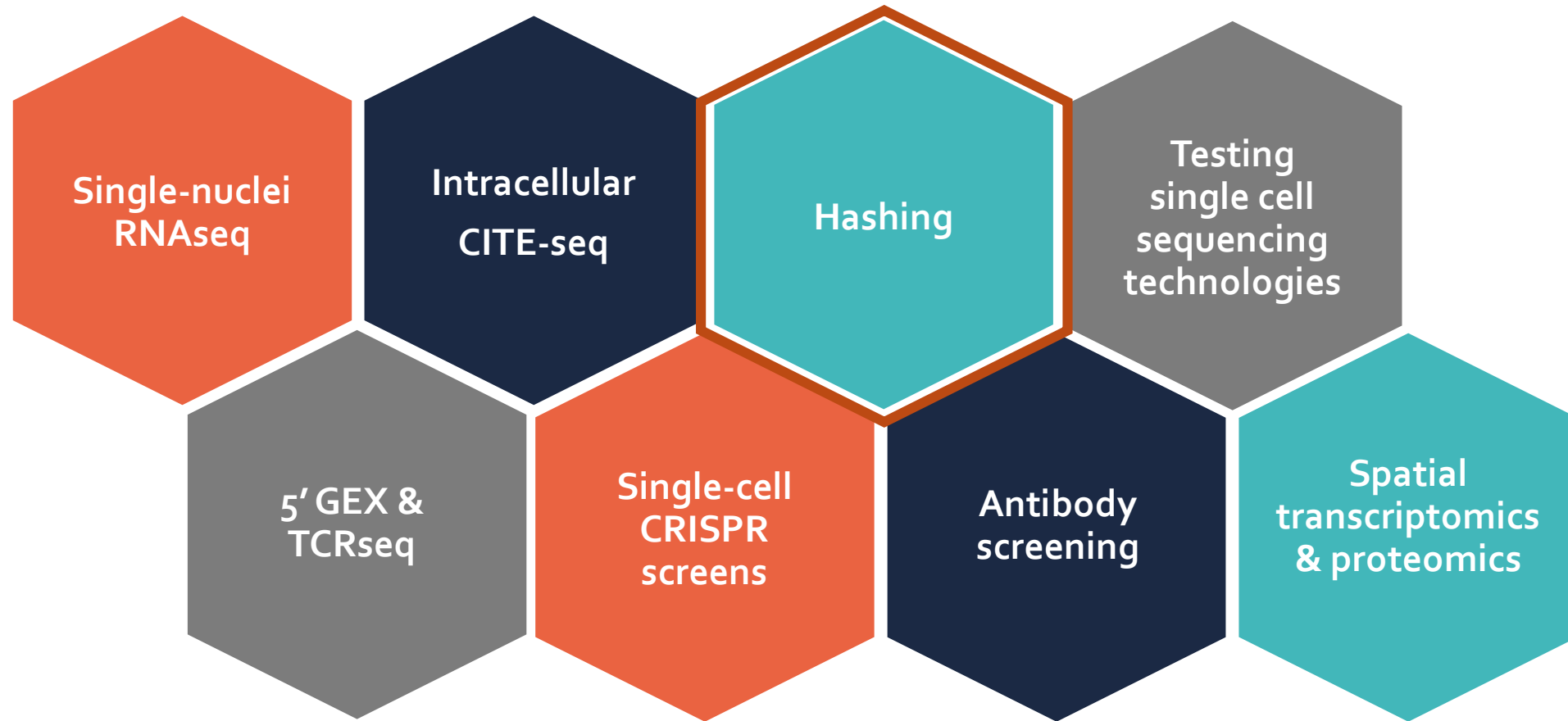


Aim:
Join forces to
test and implement emerging single cell technologies



SCIENCE MEETS LIFE

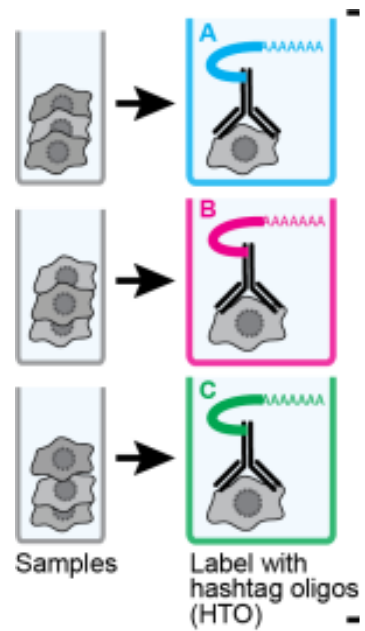
Project Plan



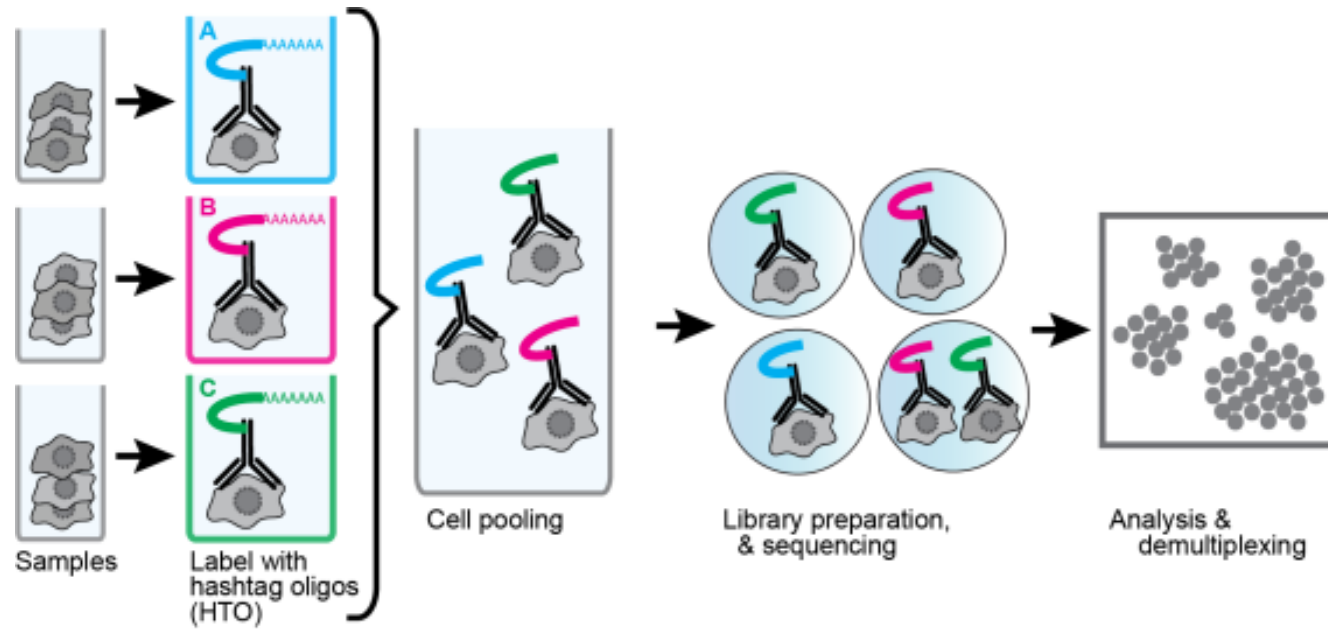
Agenda

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

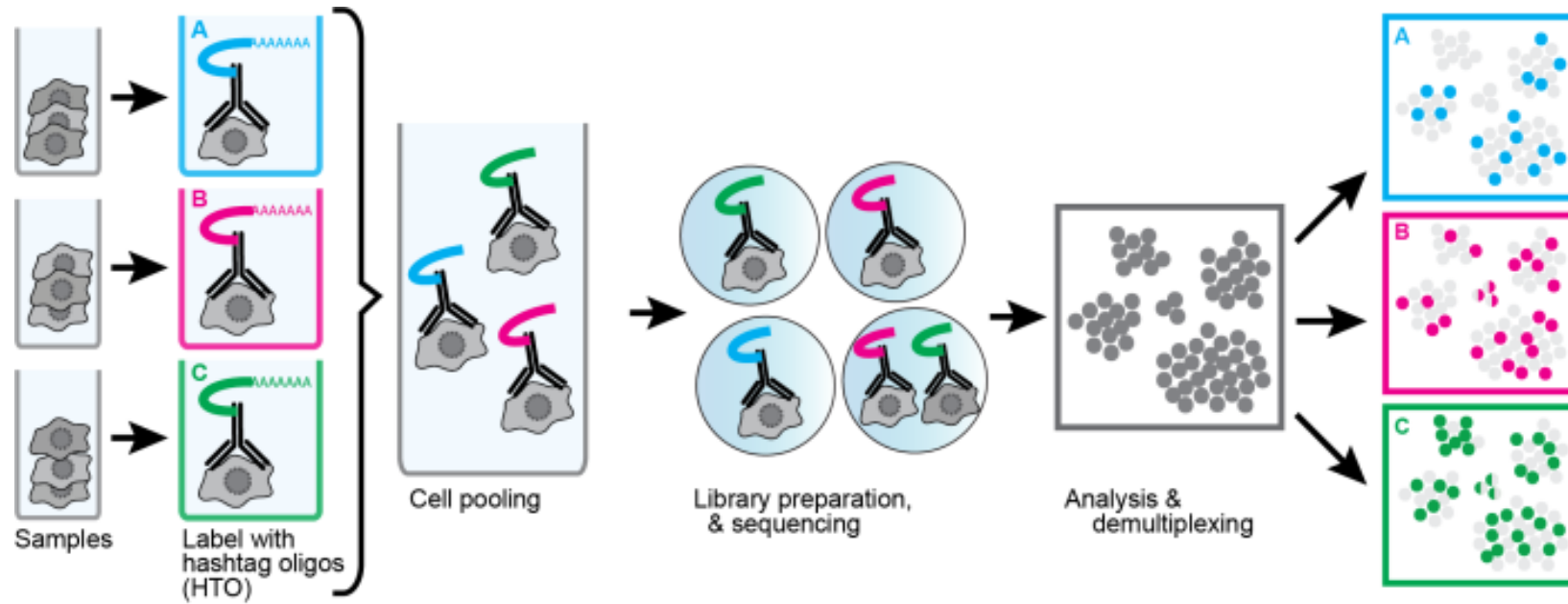
Sample multiplexing for scRNA-seq



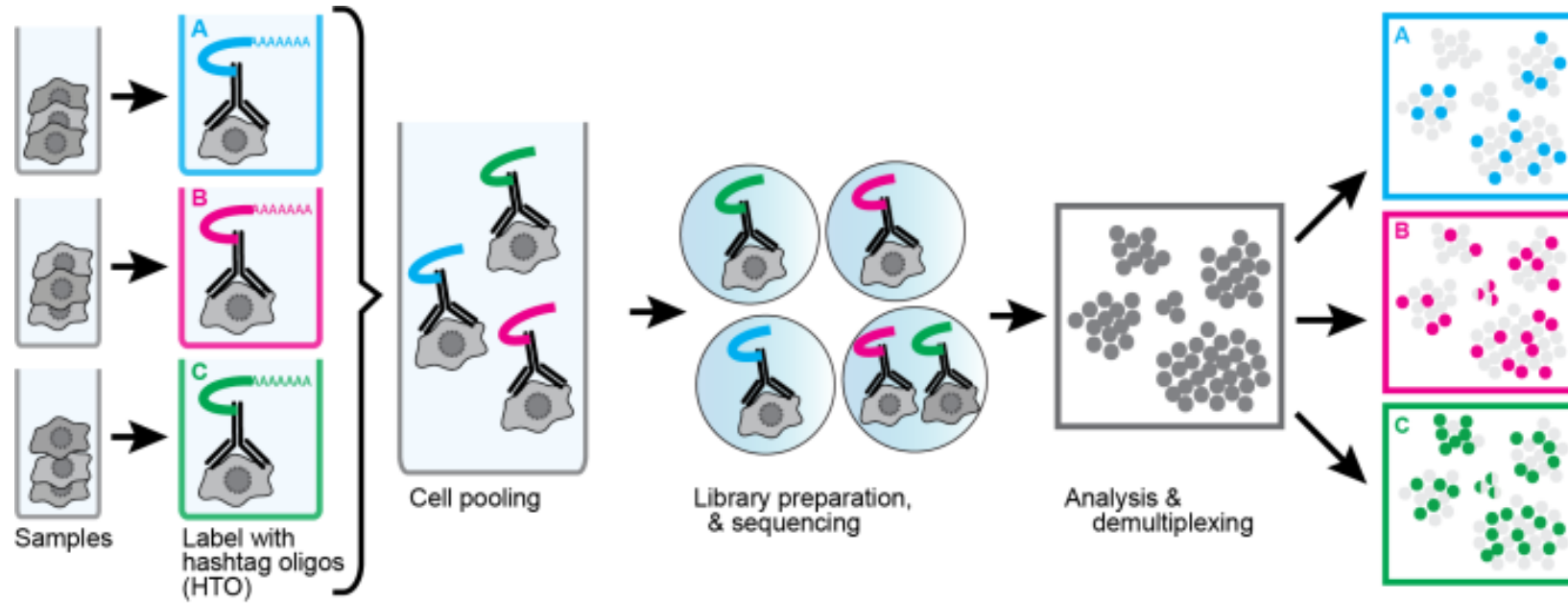
Sample multiplexing for scRNA-seq



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

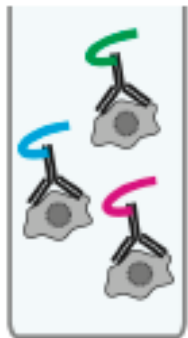
Totalseq Biolegend,
AbSeq BD

Pre-existing
variation

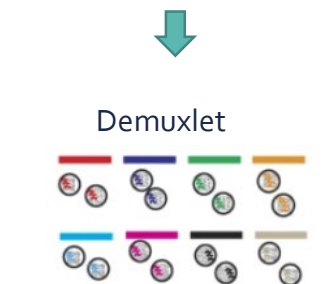
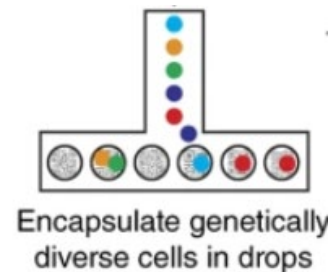
CellTag
indexing

iEDDA
click chemistry

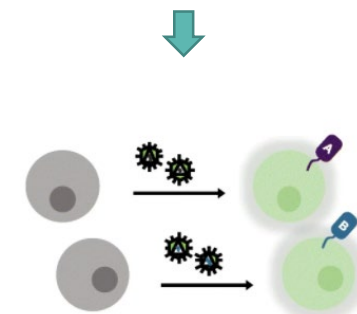
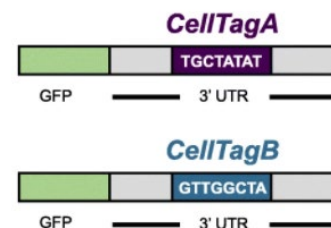
MULTIseq
lipid conjugation



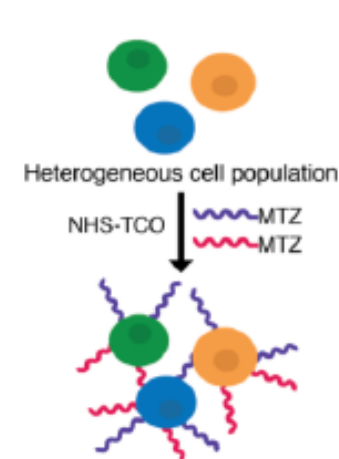
Stoeckius et al. 2018
Gaublomme et al. 2019



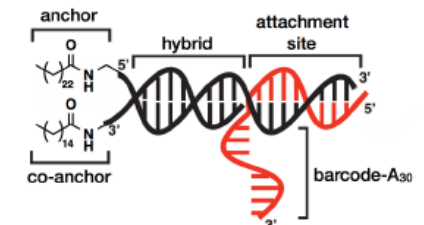
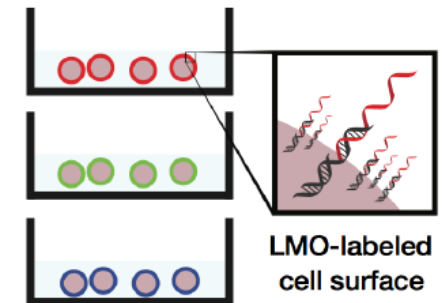
Kang et al. 2018



Guo et al. 2019



Gehring et al. 2018



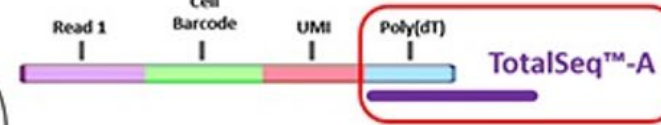
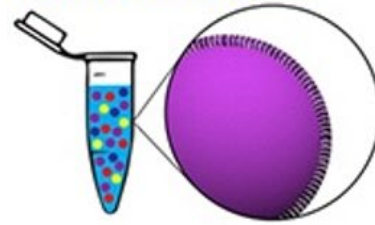
McGinnis et al. 2019

Antibody hashing of human and mouse samples

TotalSeq™-A



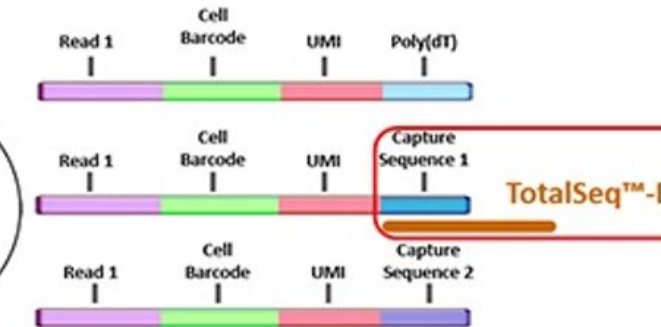
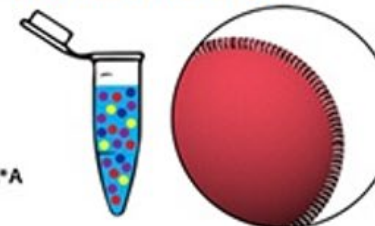
Poly(dT)-based Systems



TotalSeq™-B



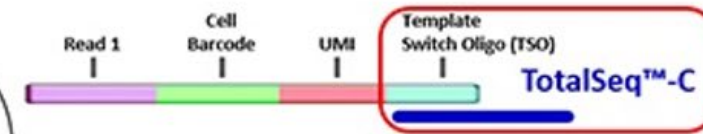
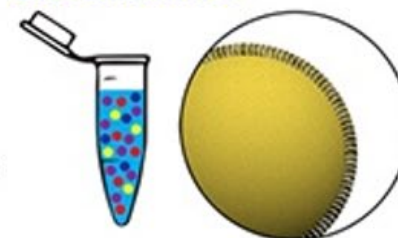
10x Genomics 3' v3



TotalSeq™-C



10x Genomics 5'

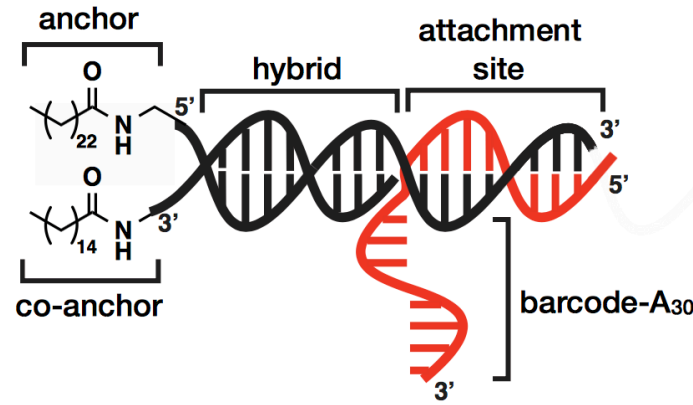


Lipid-modified oligo hashing

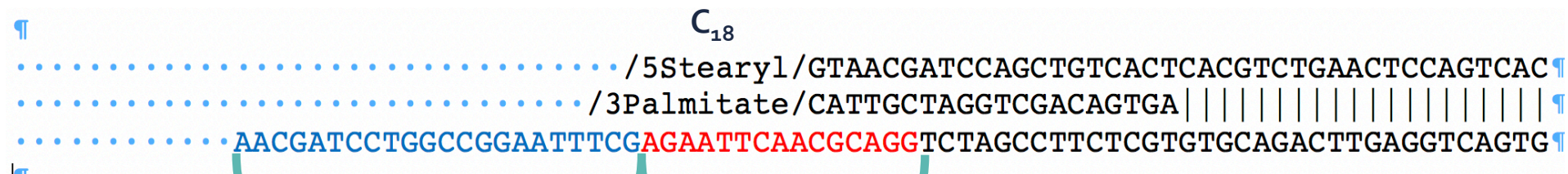
1. MULTI-seq LMO for cells

2. CMO for nuclei

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

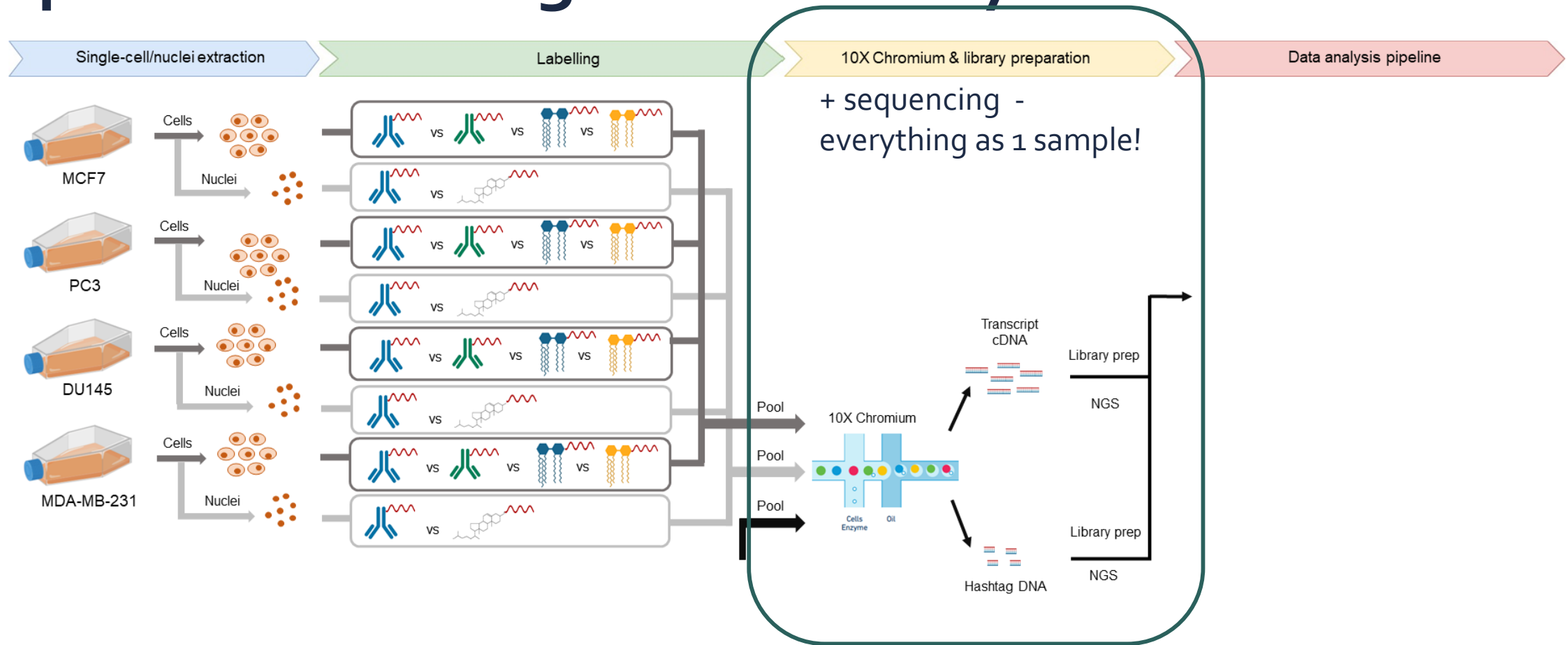


(McGinnis et al;
Nature Methods; 2019)



10x Feature barcode Hashtag Barcode

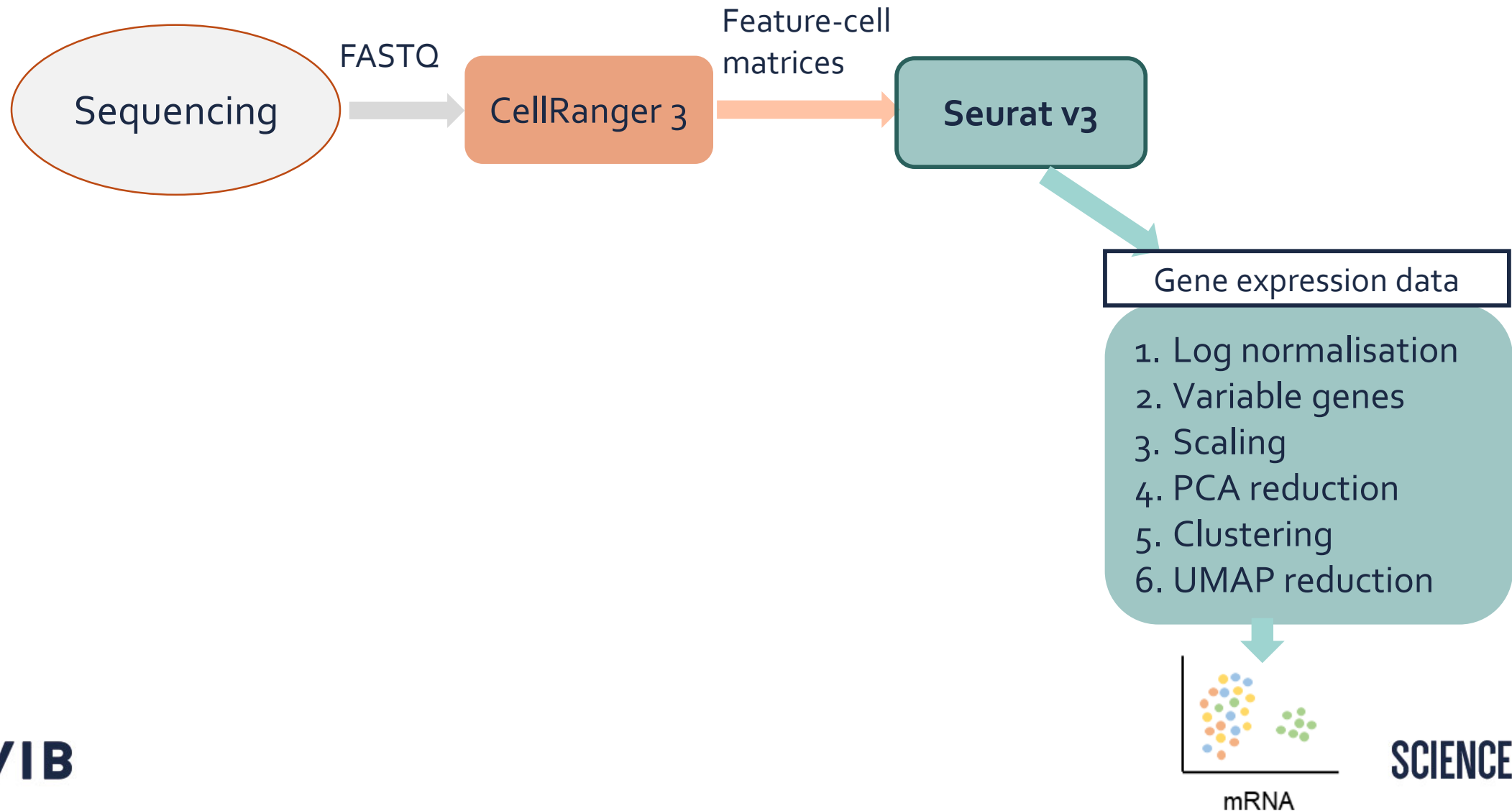
Experimental design of the study



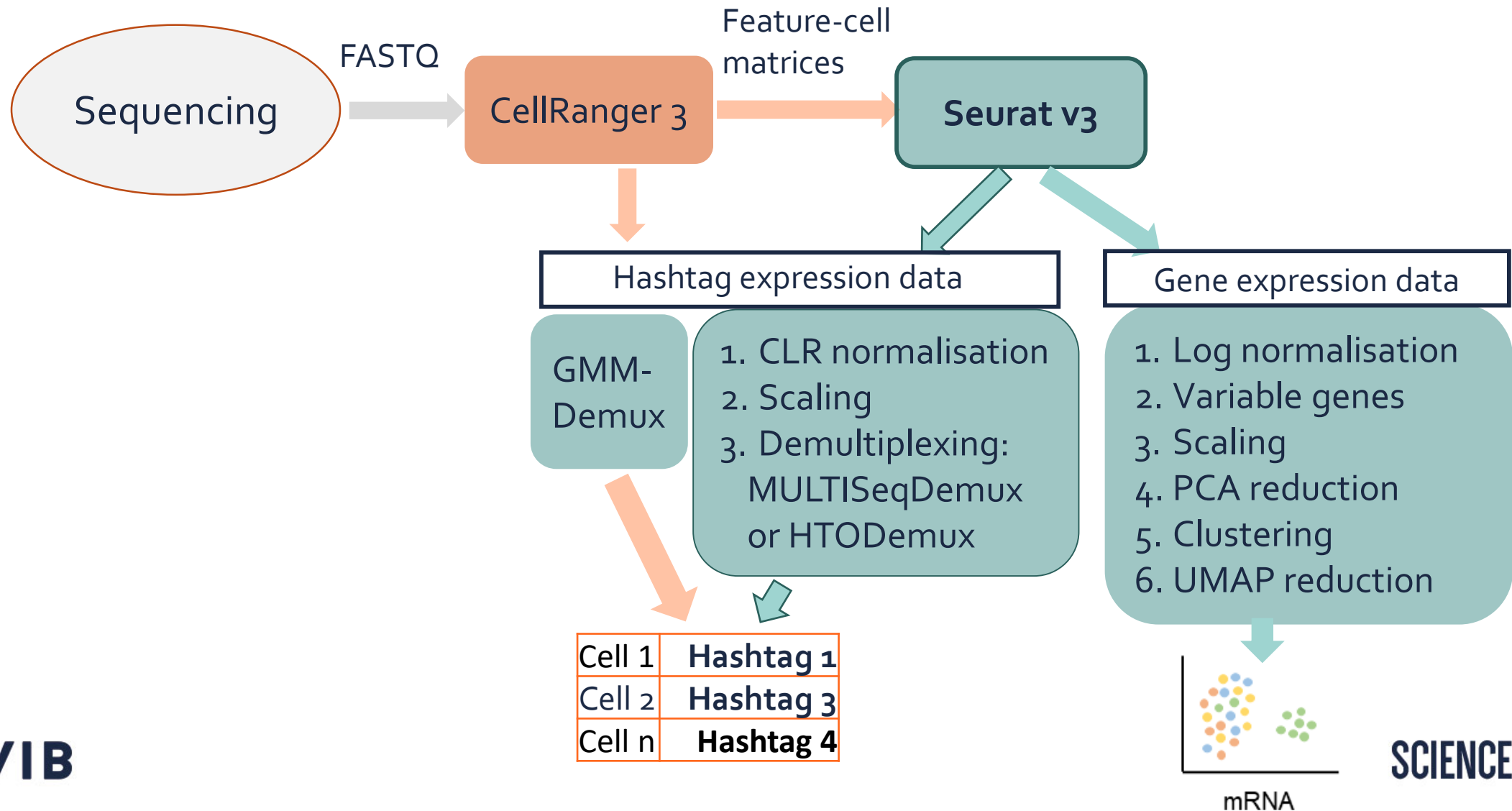
Analysis pipeline of the study



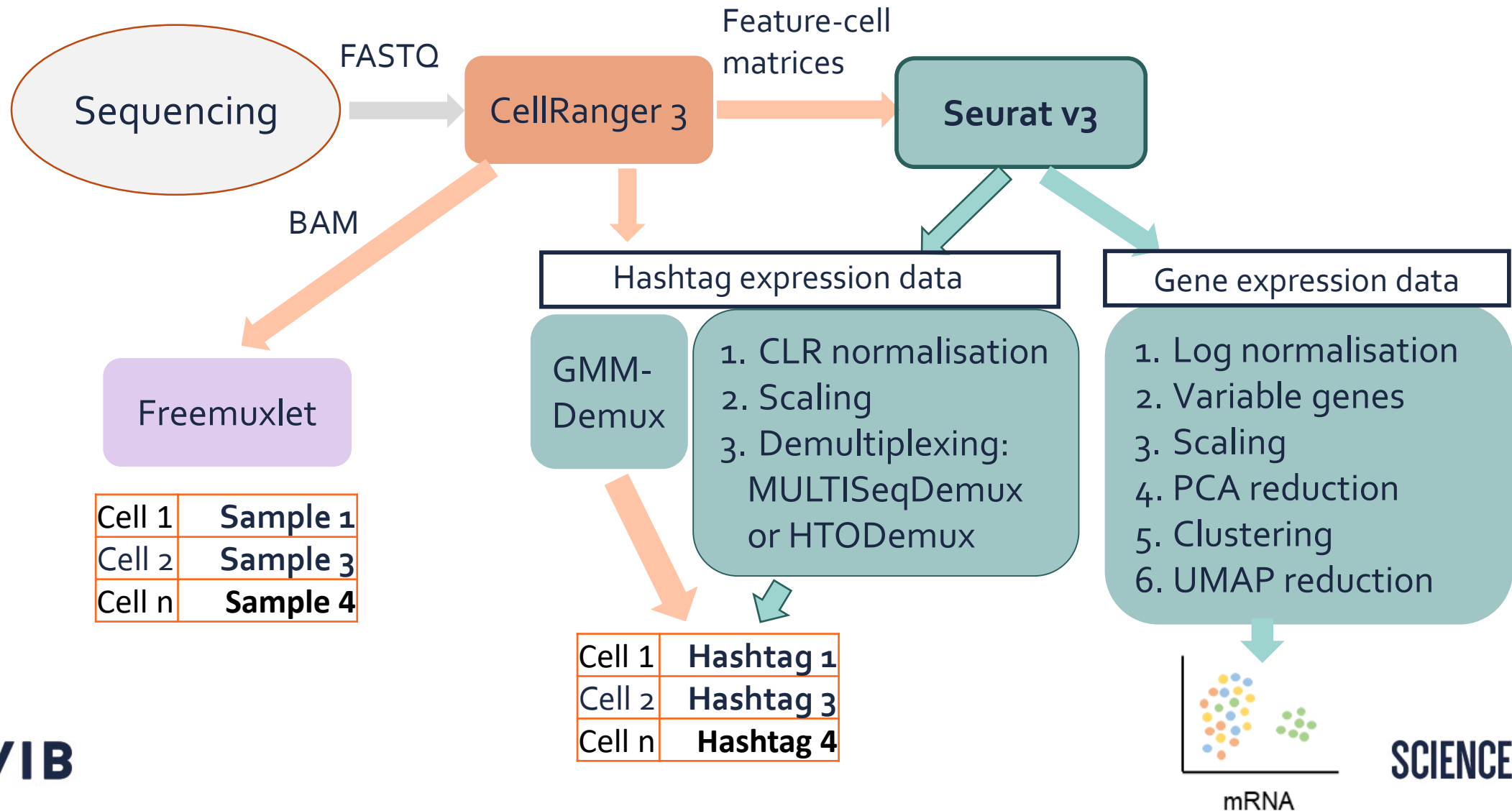
Analysis pipeline of the study



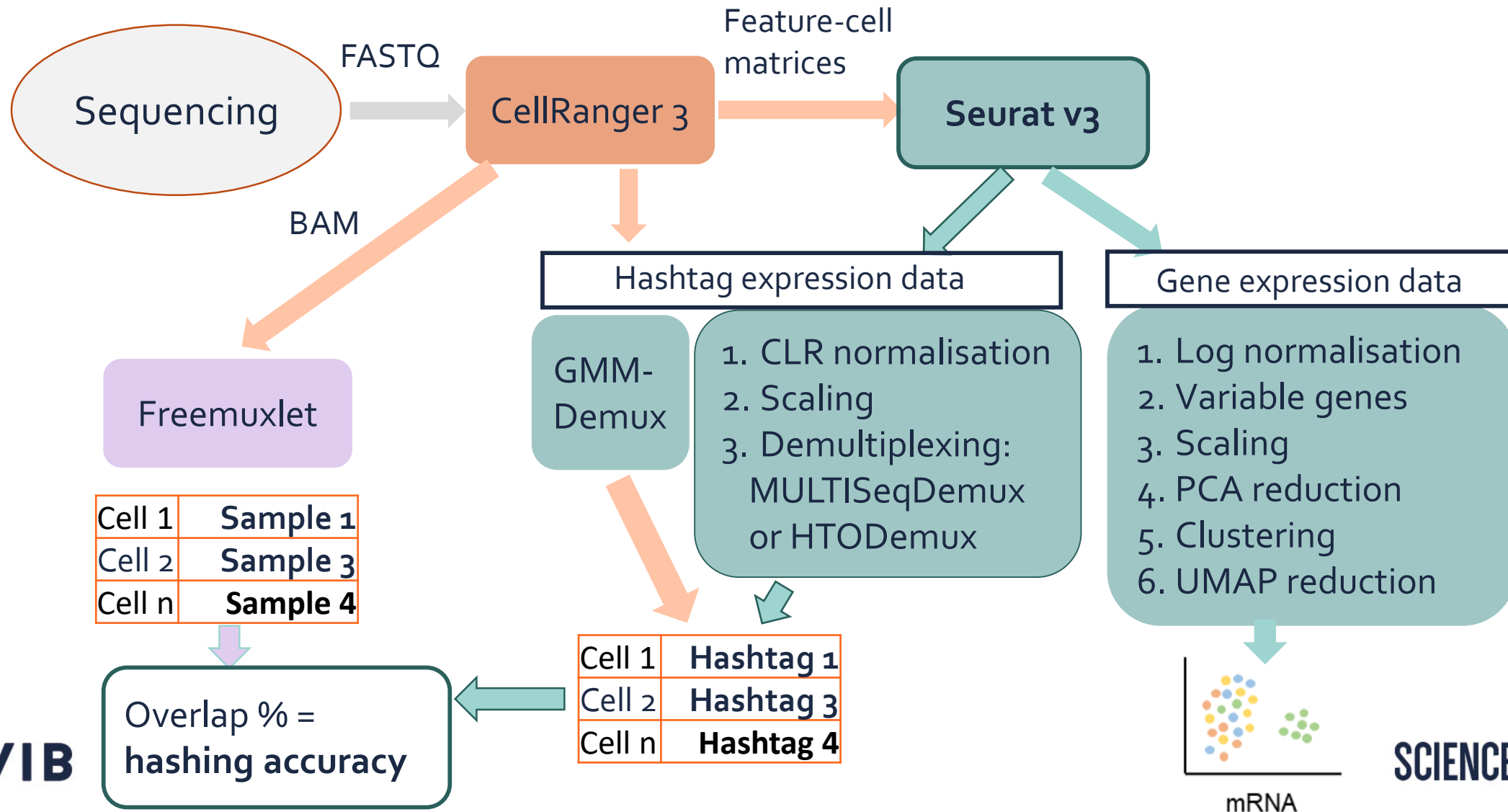
Analysis pipeline of the study



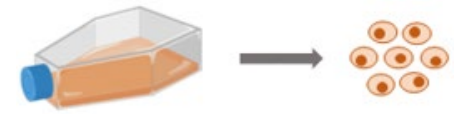
Analysis pipeline of the study



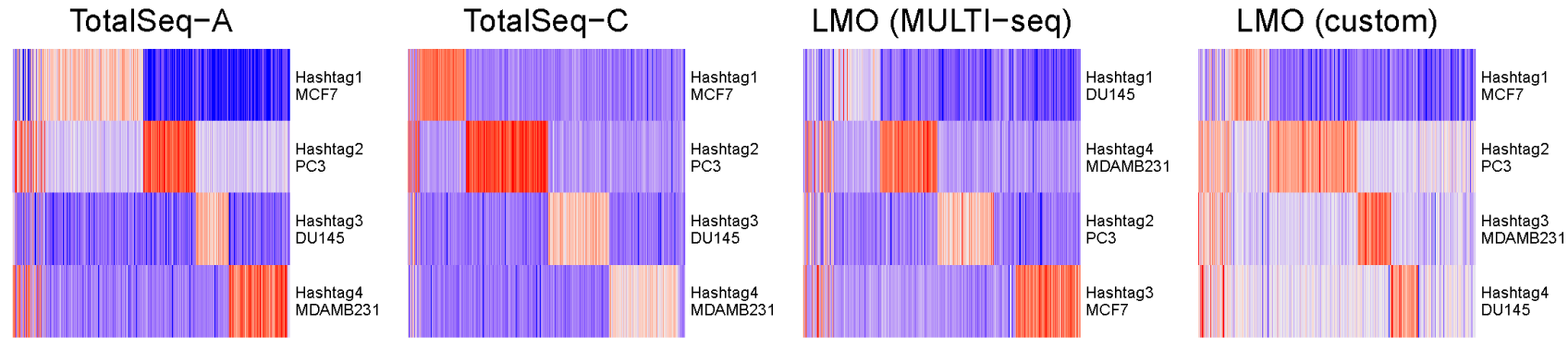
Analysis pipeline of the study



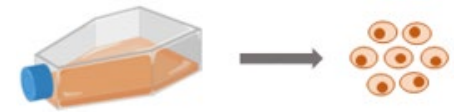
Antibody vs lipid cell hashing



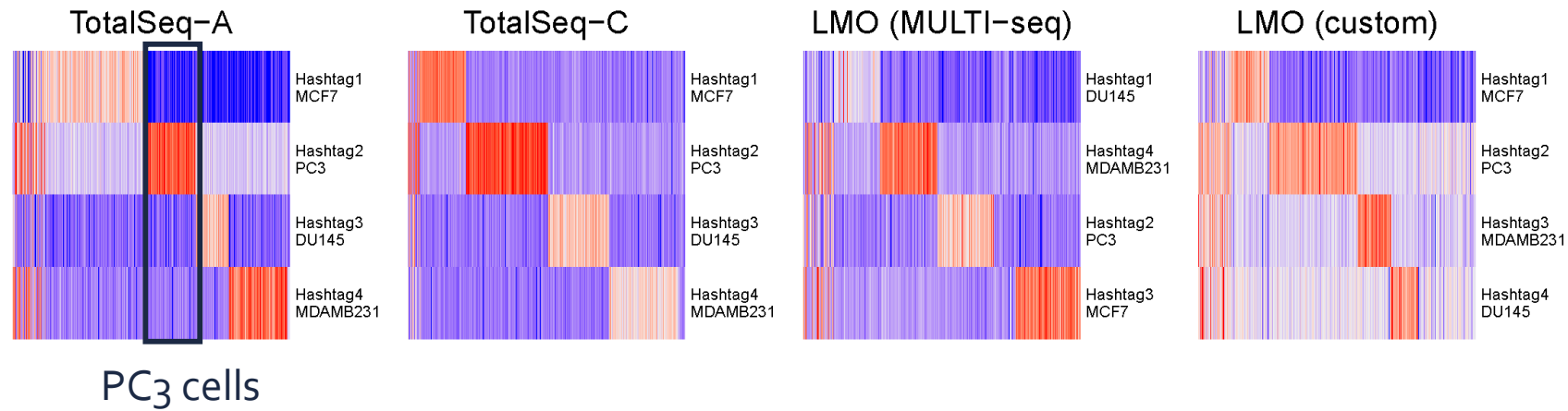
Log-transformed hashtag counts



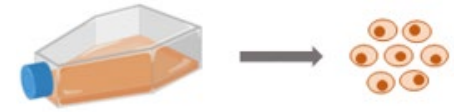
Antibody vs lipid cell hashing



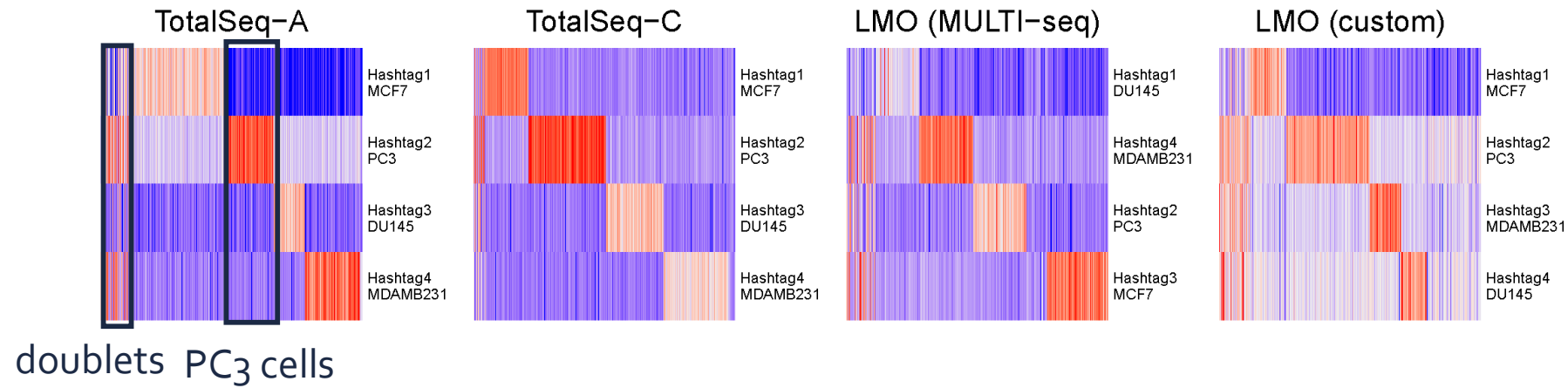
Log-transformed hashtag counts



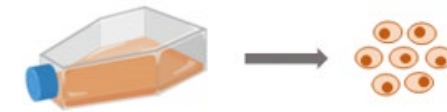
Antibody vs lipid cell hashing



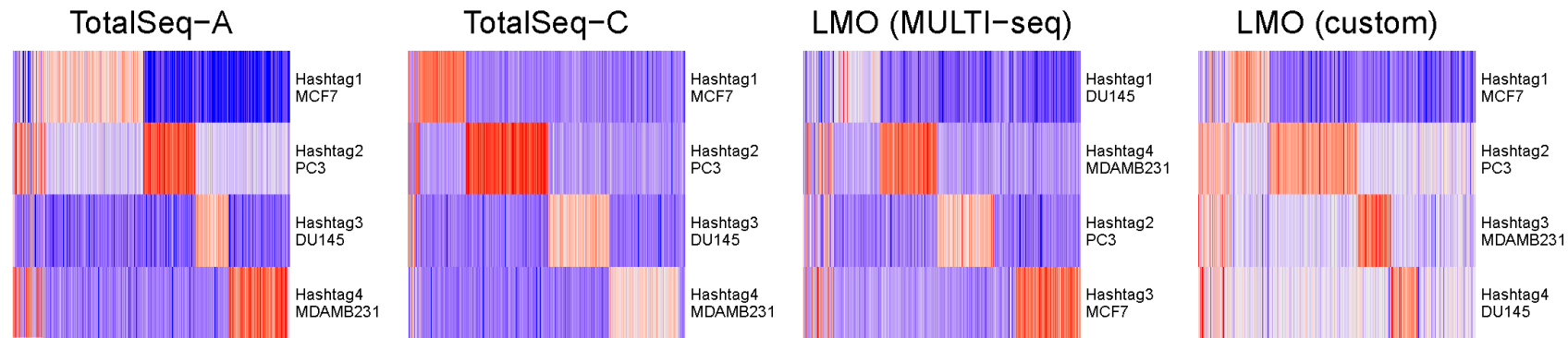
Log-transformed hashtag counts



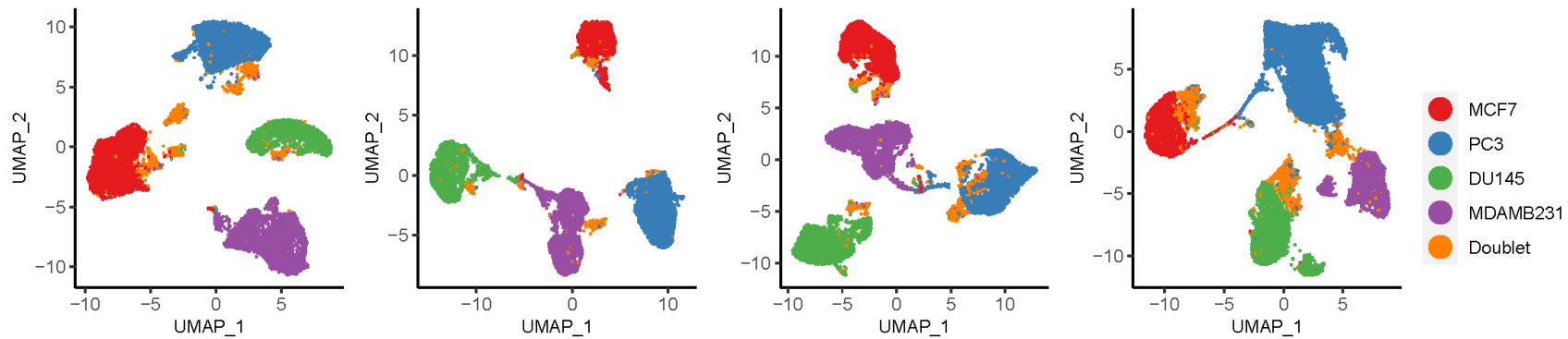
Antibody vs lipid cell hashing



Log-transformed hashtag counts



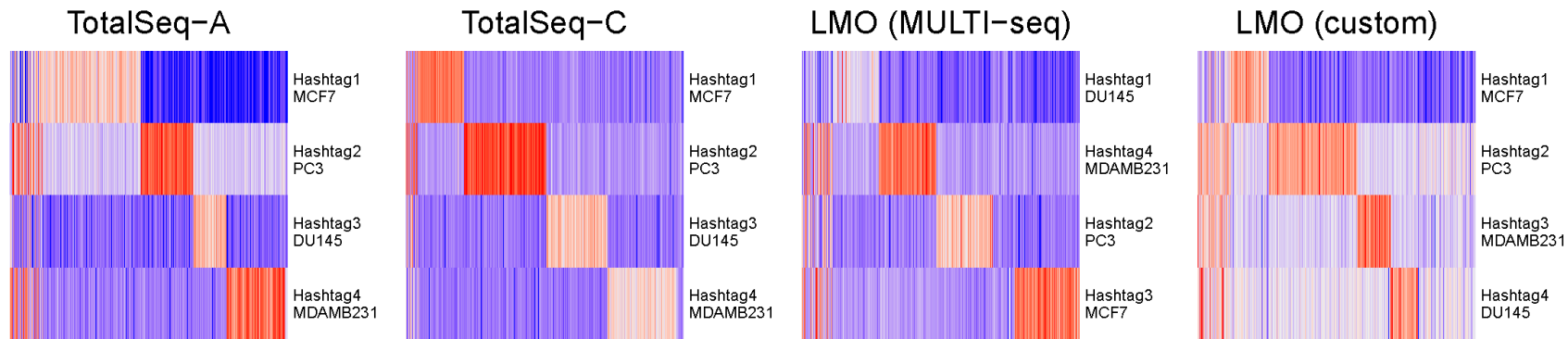
Freemuxlet annotation



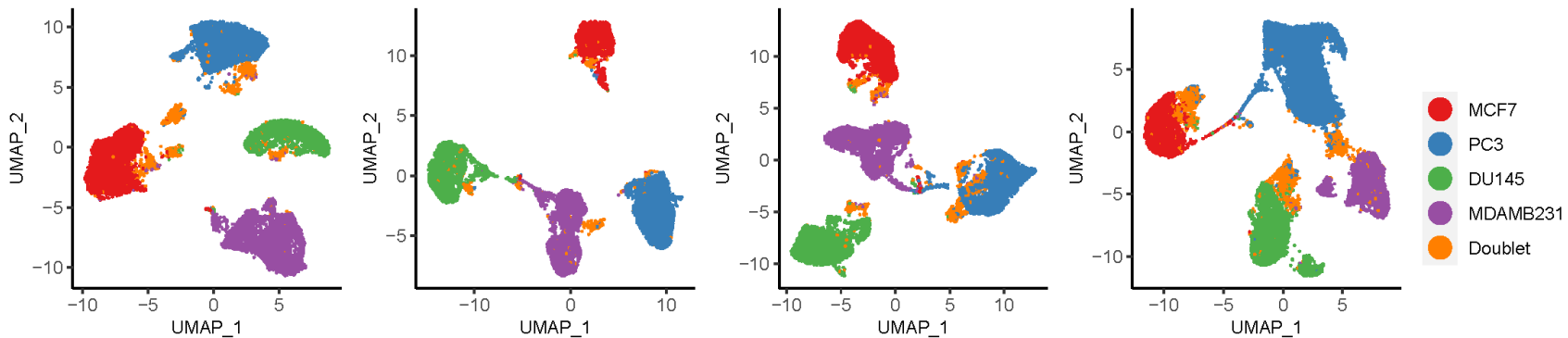
Antibody vs lipid cell hashing



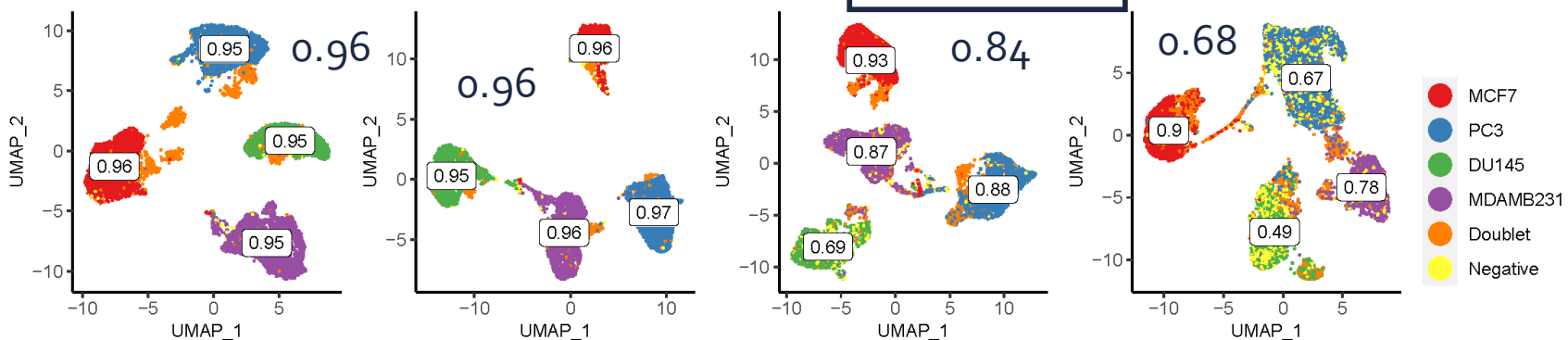
Log-transformed hashtag counts



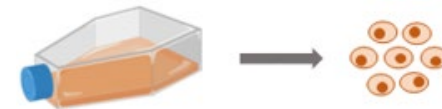
Freemuxlet annotation



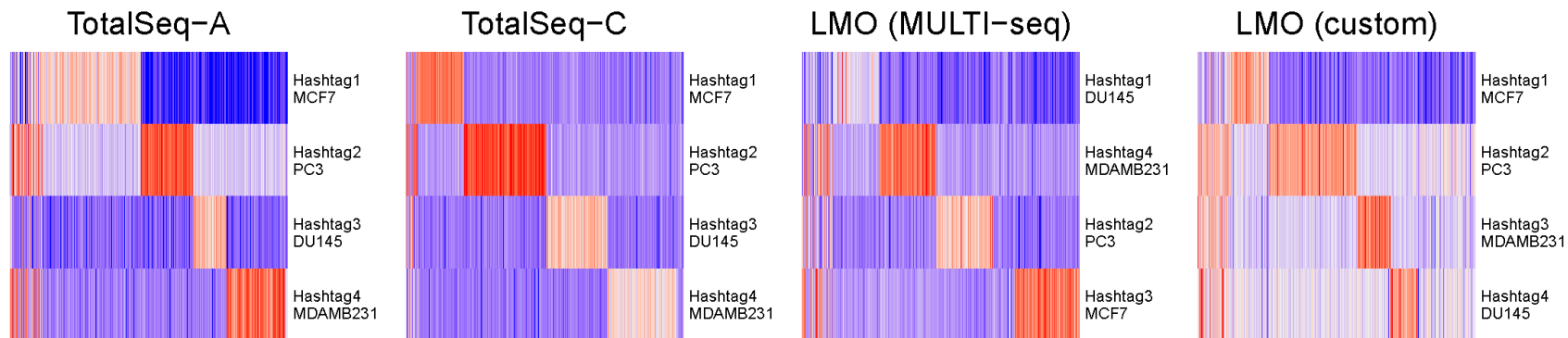
MULTISeqDemux annotation and classification accuracy



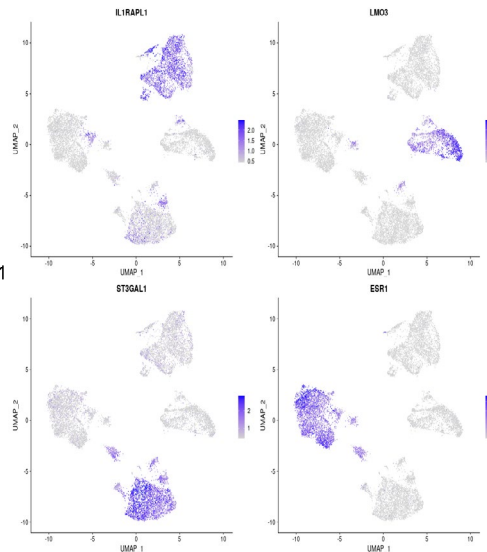
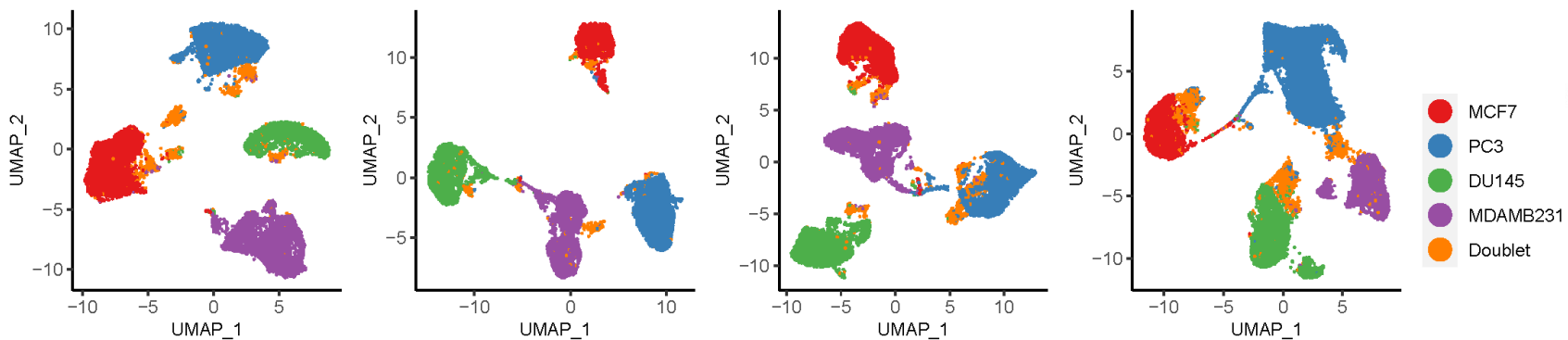
Antibody vs lipid cell hashing



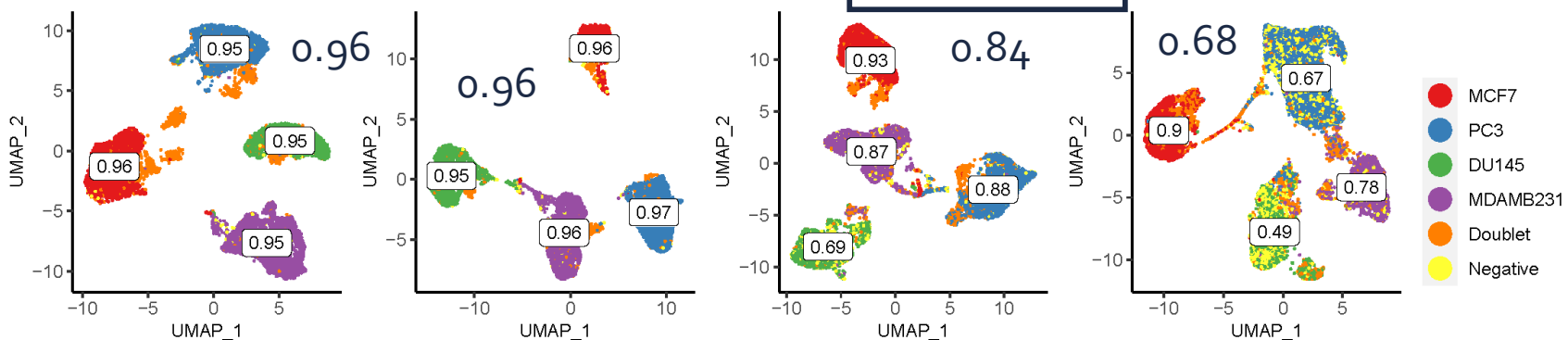
Log-transformed hashtag counts



Freemuxlet annotation

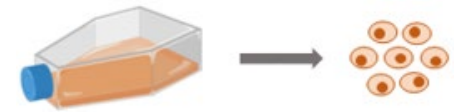


MULTISeqDemux annotation and classification accuracy

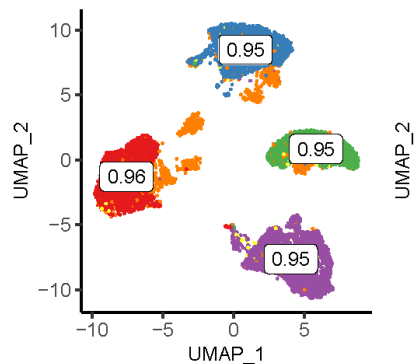
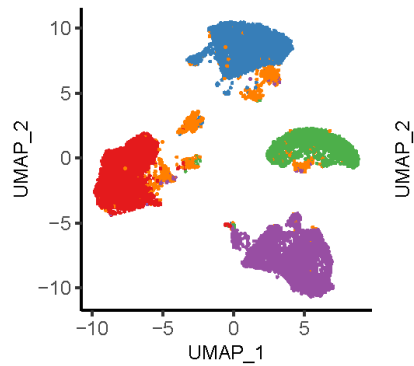
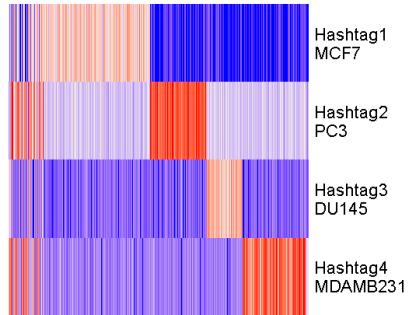


Antibody vs lipid cell hashing

Log-transformed hashtag counts



TotalSeq-A

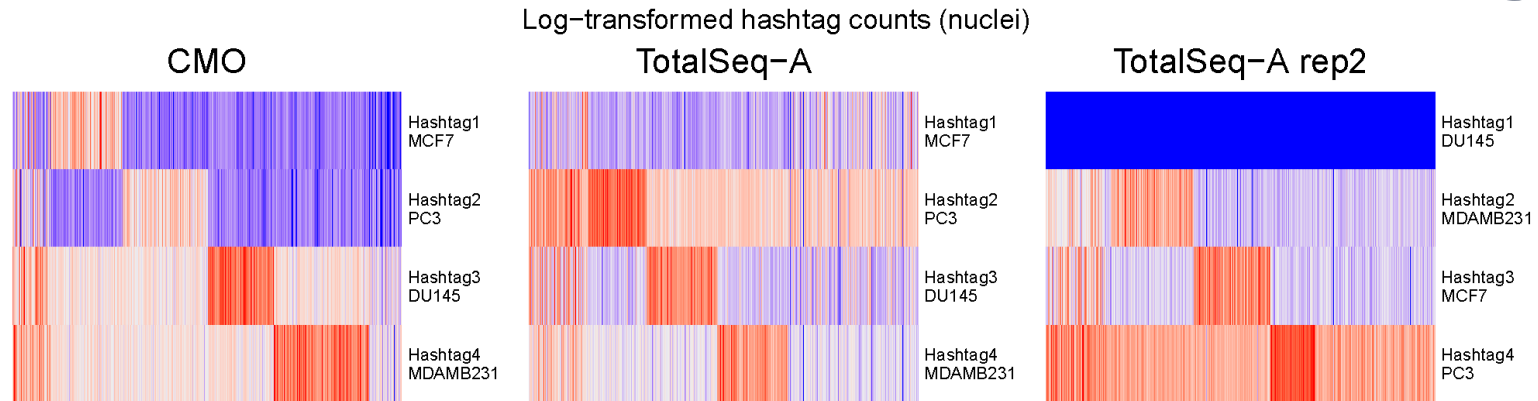
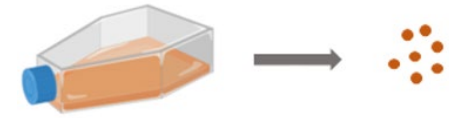


Experiment	TotalSeq A cell	TotalSeq A cell rep2
Number of Cells (CellRanger)	11869	17611
Hashing accuracy (MULTISeqDemux)	0.96	0.91

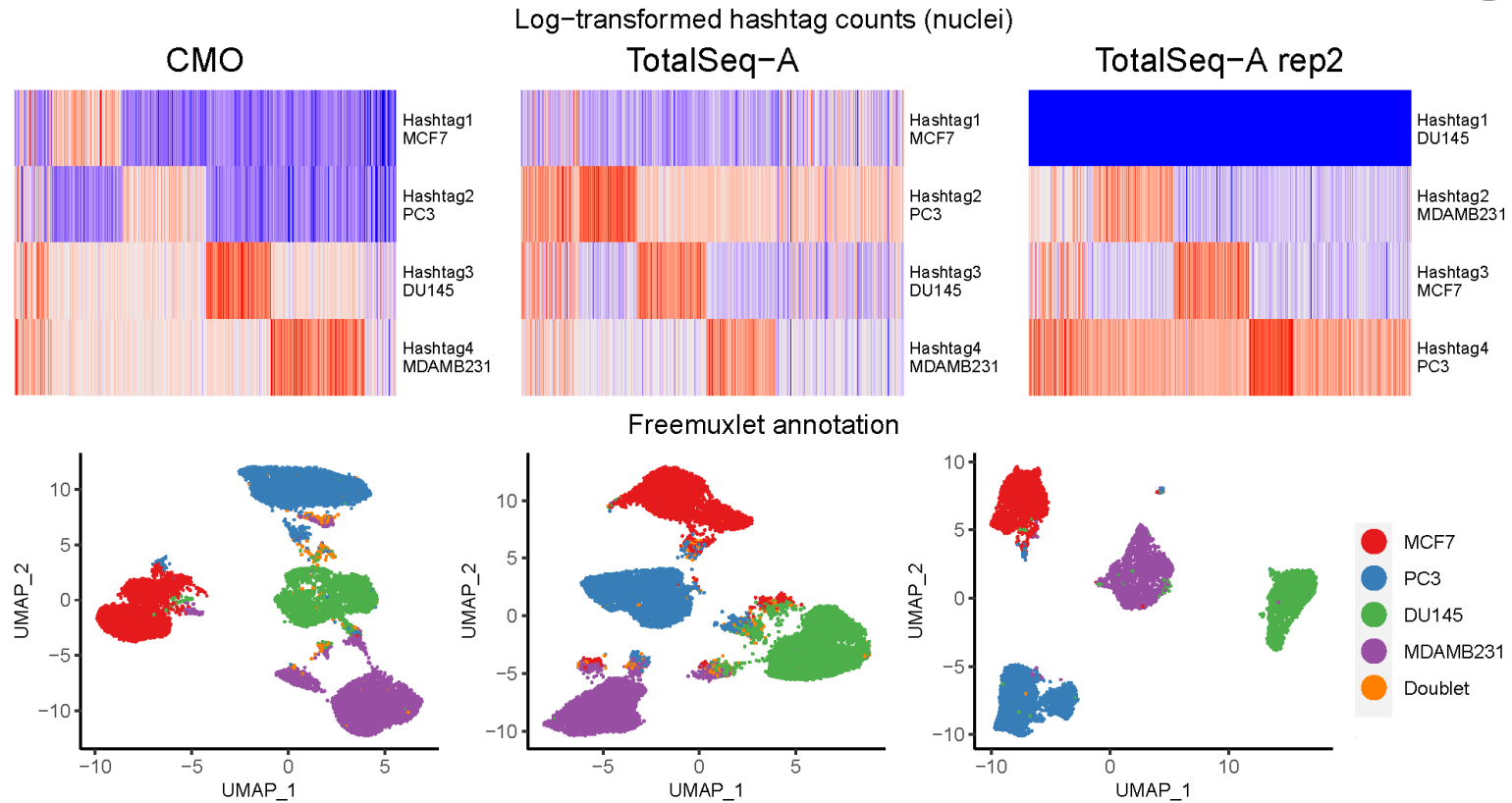
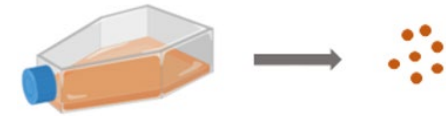


48% increase in captured cells, but 125% more doublets and 325% increase in negatives

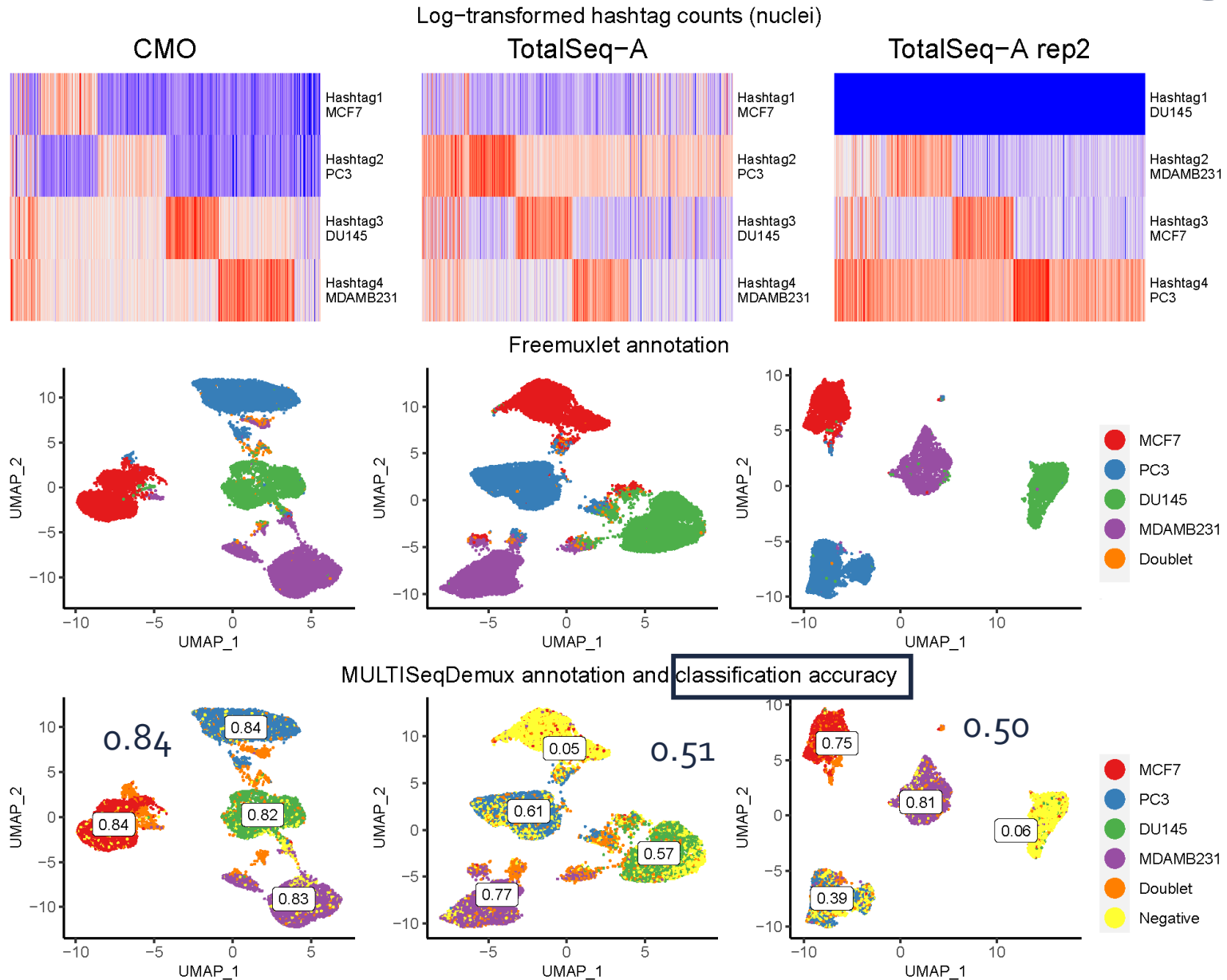
Cholesterol vs antibody nuclei hashing



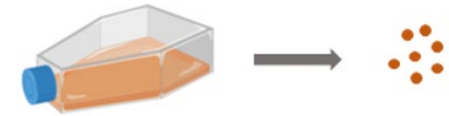
Cholesterol vs antibody nuclei hashing



Cholesterol vs antibody nuclei hashing



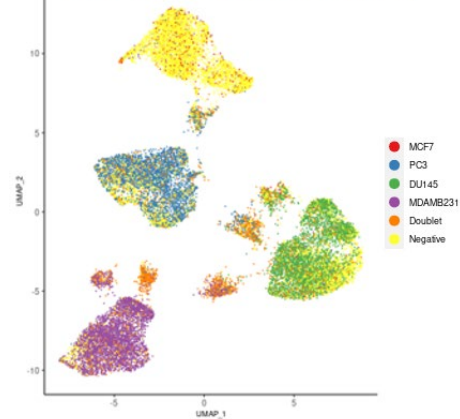
Cholesterol vs antibody nuclei hashing



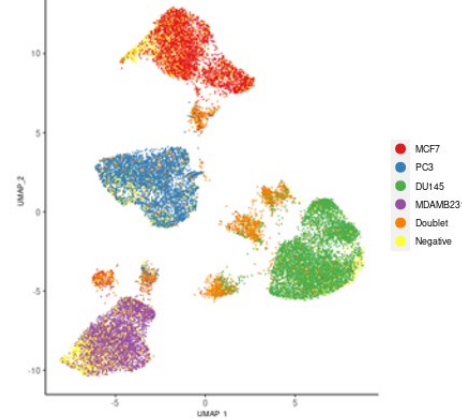
Finetuned demultiplexing

Finetuned demultiplexing

MULTISeqDemux (autoTresh=T)



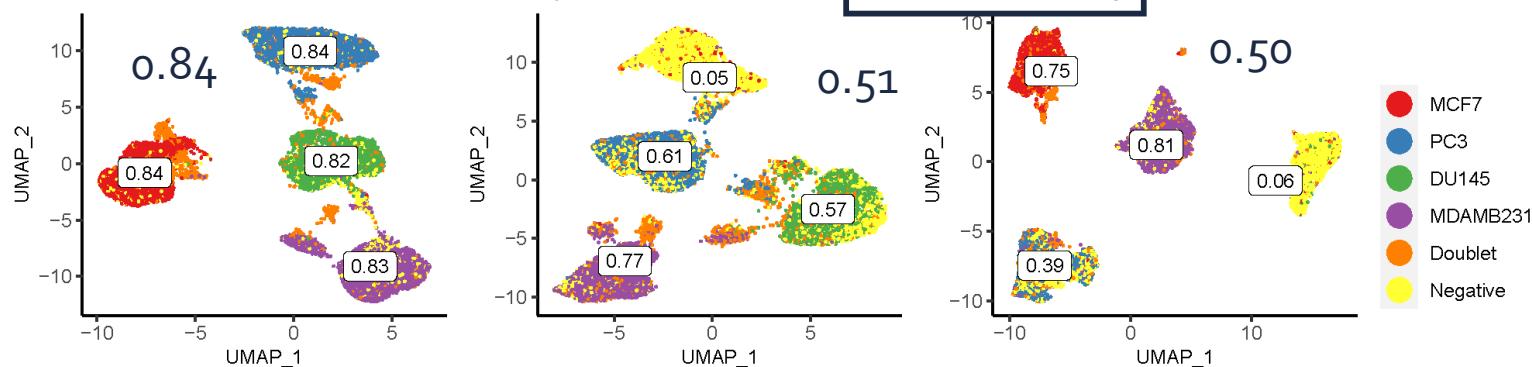
HTODemux (pos.quantile=0.9)

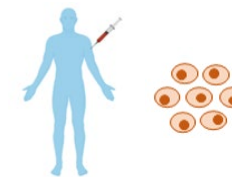


Experiment	TotalSeq A nuclei	TotalSeq A nuclei
Number of Cells (CellRanger)	23451	23451
Hashing accuracy (MULTISeqDemux)	0.51	0.65



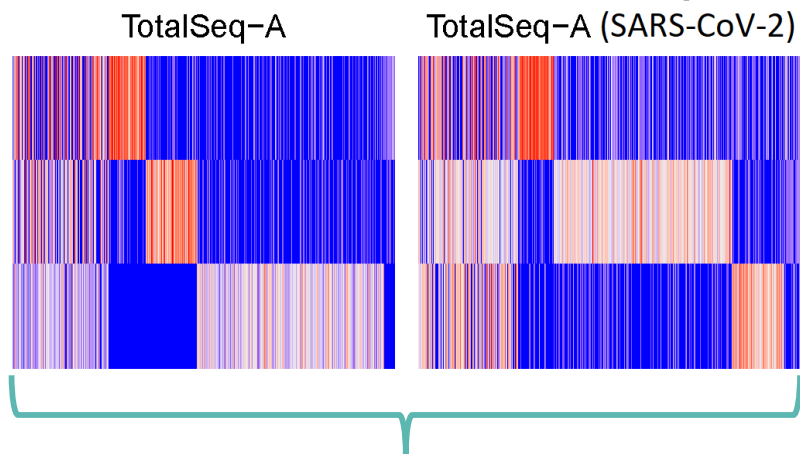
MULTISeqDemux annotation and classification accuracy



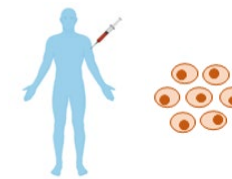


Human PBMC hashing

Log-transformed hashtag counts

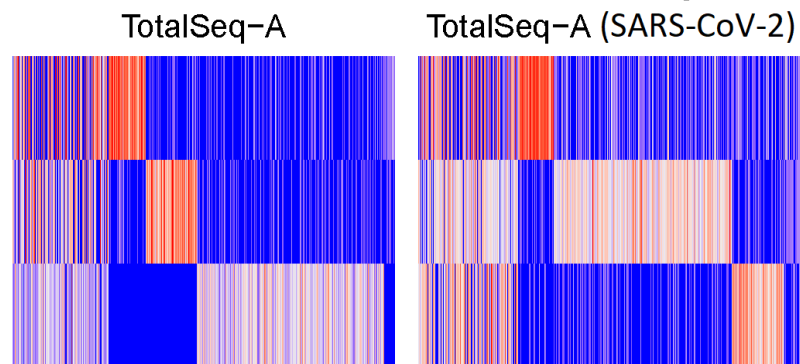


Additional CITE-seq panel of 277 TotalSeq A antibodies

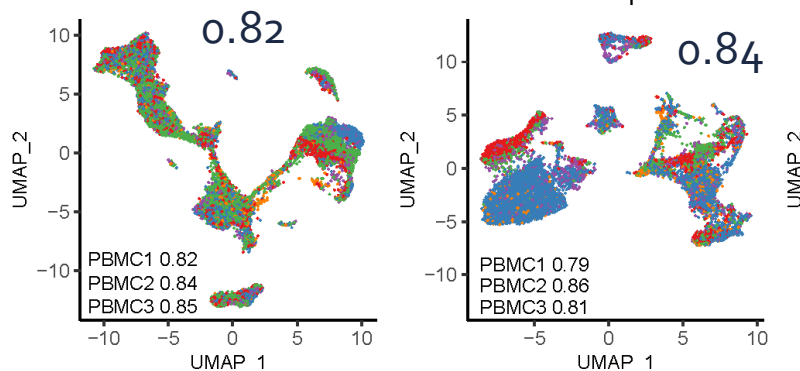


Human PBMC hashing

Log-transformed hashtag counts

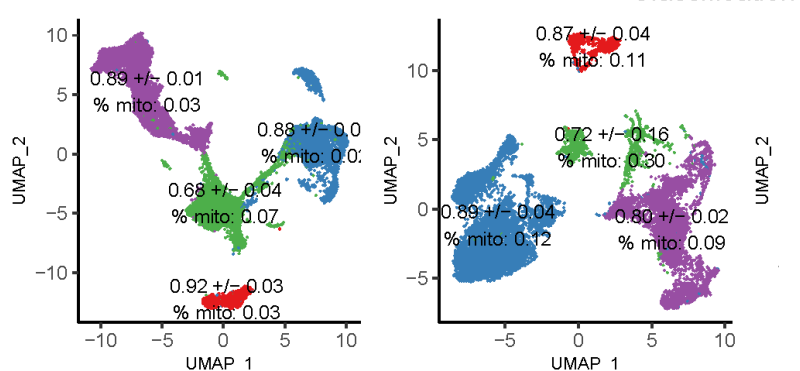


MULTISeqDemux annotation and classification accuracy



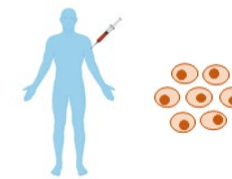
- PBMC1
- PBMC2
- PBMC3
- Doublet
- Negative

Classification accuracy per cell type



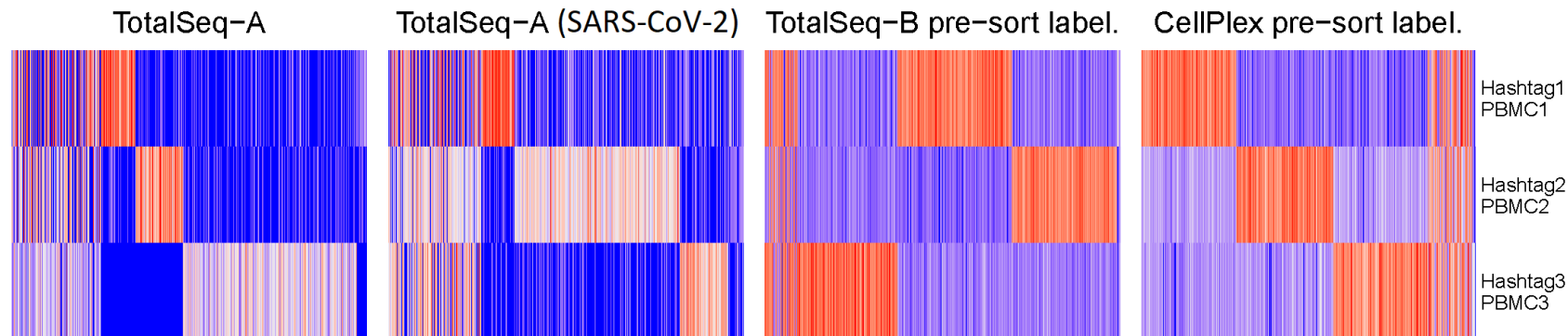
- B/Plasma cells
- Monocytes/DCs
- Other
- T/NK cells

Human PBMC hashing

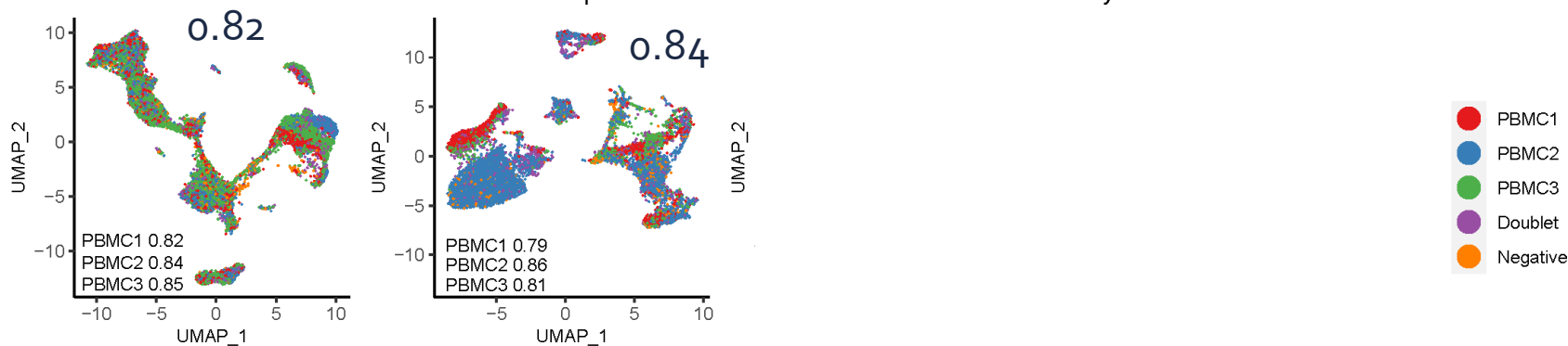


10X
GENOMICS®

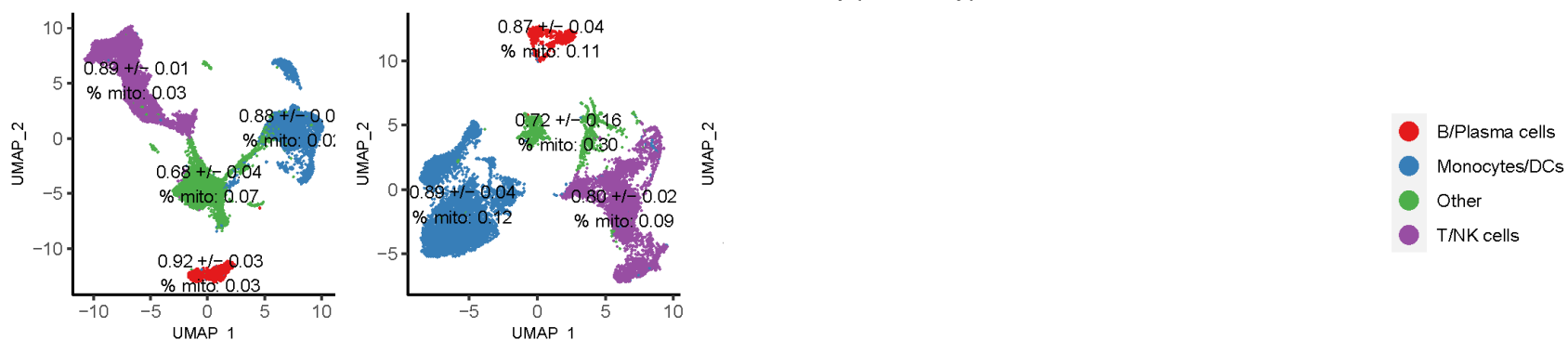
Log-transformed hashtag counts



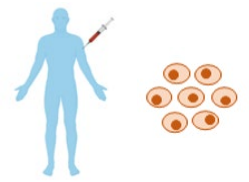
MULTISeqDemux annotation and classification accuracy



Classification accuracy per cell type



Human PBMC hashing



10X
GENOMICS®

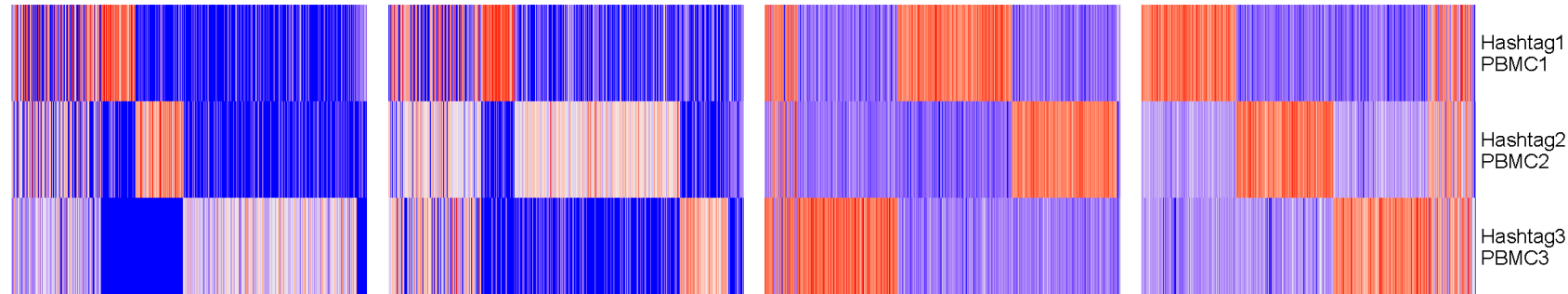
Log-transformed hashtag counts

TotalSeq-A

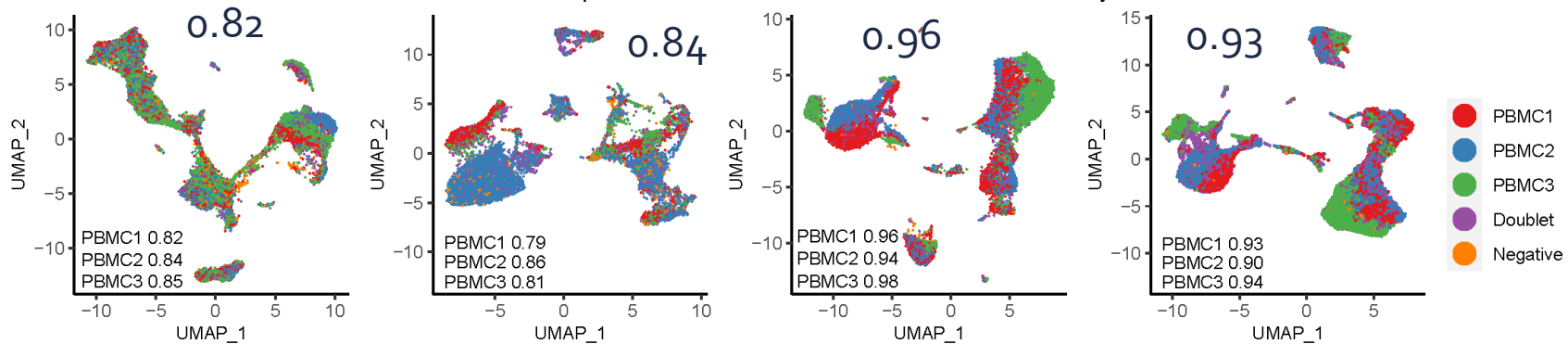
TotalSeq-A (SARS-CoV-2)

TotalSeq-B pre-sort label.

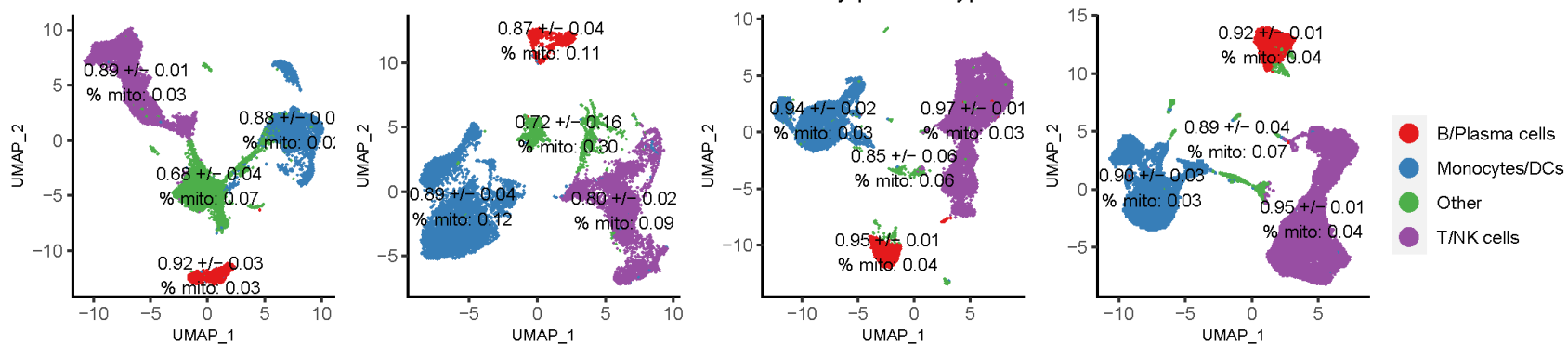
CellPlex pre-sort label.



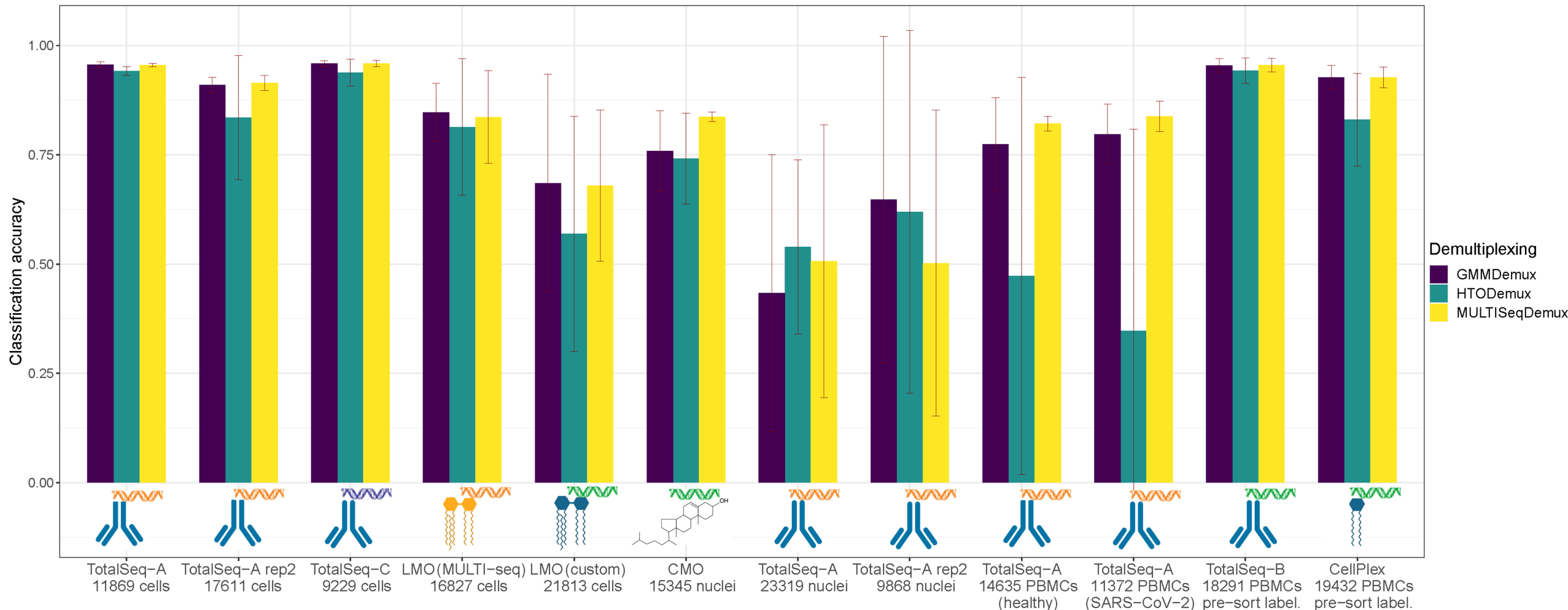
MULTISeqDemux annotation and classification accuracy



Classification accuracy per cell type



Overview of human cell and nuclei hashing



Mice tissue hashing

Log-transformed hashtag counts

TotalSeq-B pre-sort label. LMO (MULTISEq) pre-sort label. LMO (custom) pre-sort label.



BALB/c



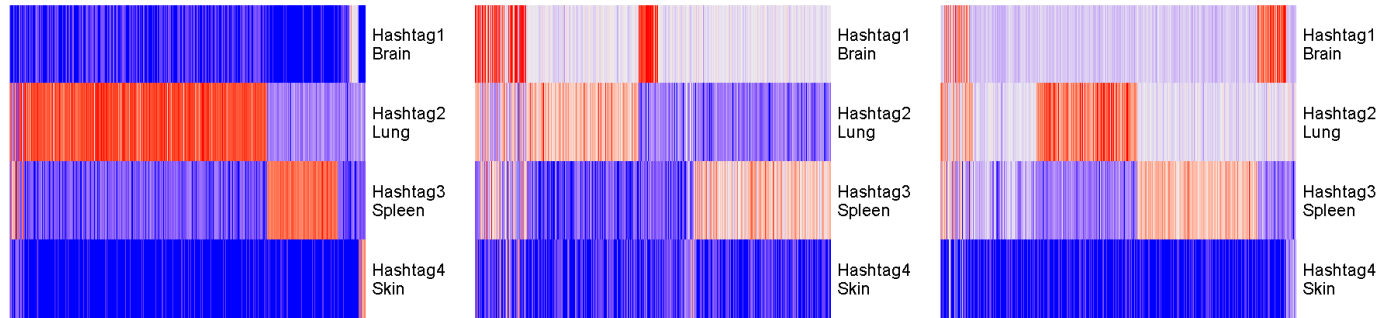
C57BL6J



C57BL6N



129SVEvs7



Mice tissue hashing

Log-transformed hashtag counts

TotalSeq-B pre-sort label. LMO (MULTISeq) pre-sort label. LMO (custom) pre-sort label.



BALB/c



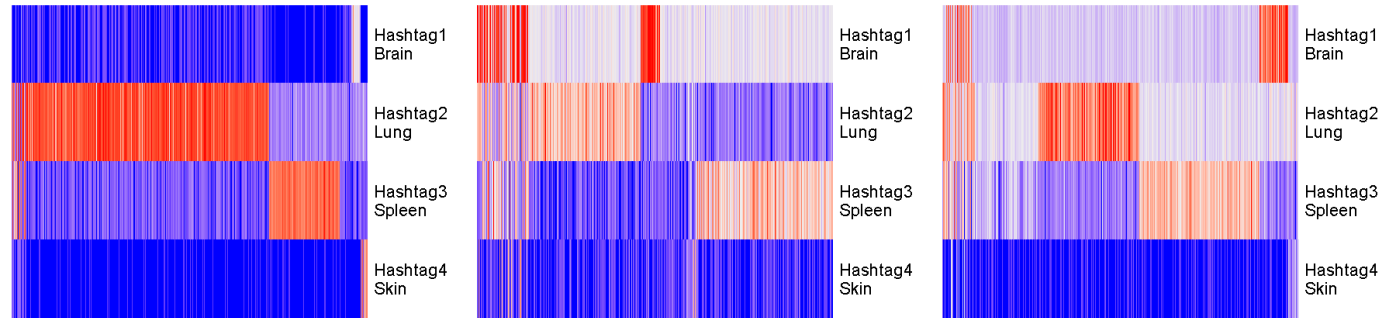
C57BL6J



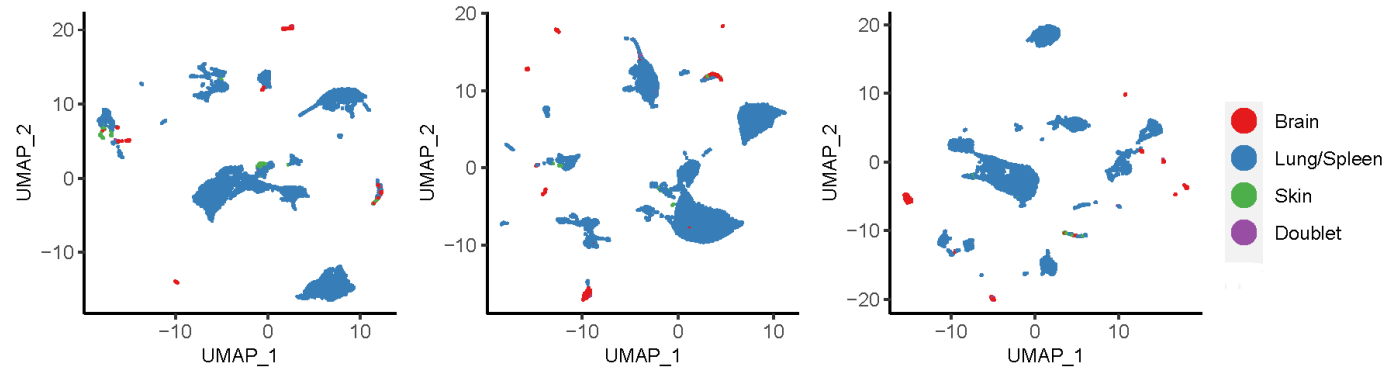
C57BL6N



129SVEvs7



Freemuxlet annotation



Mice tissue hashing



BALB/c

C57BL6J

C57BL6N

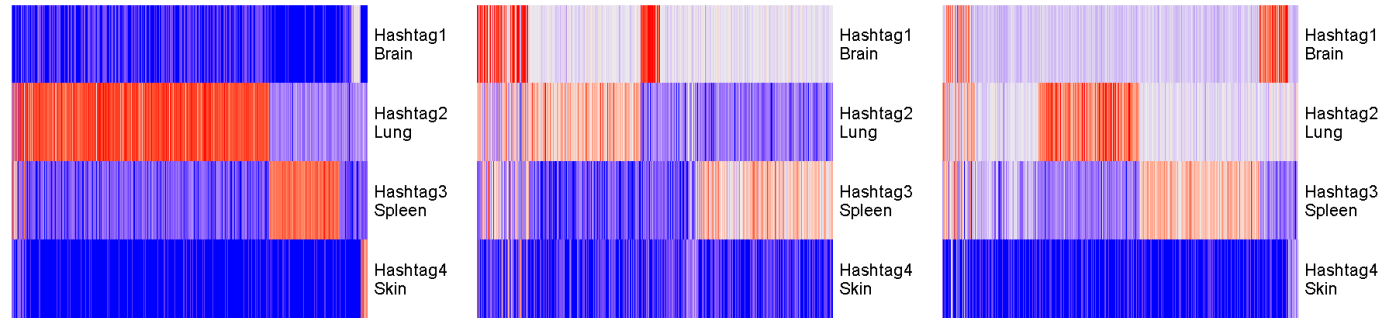
129SVEvs7

Log-transformed hashtag counts

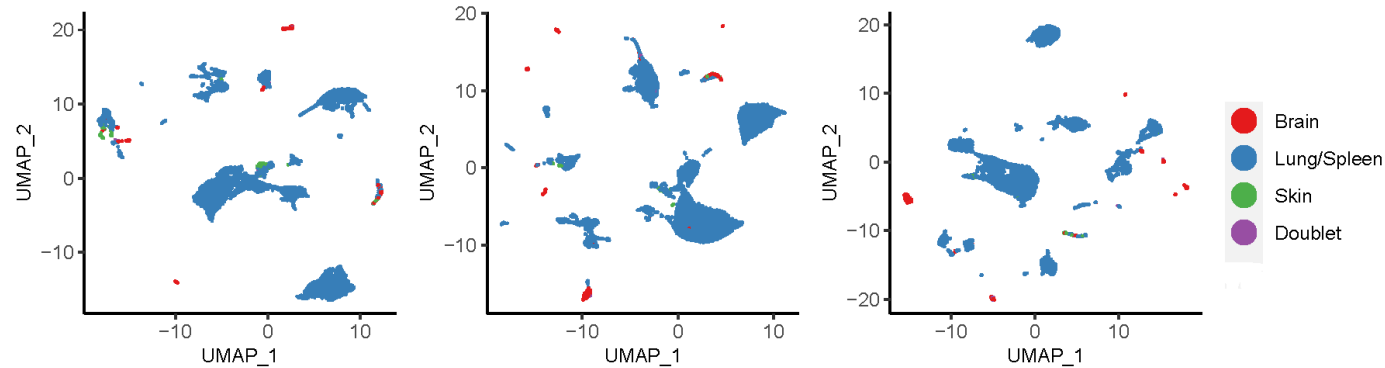
TotalSeq-B pre-sort label.

LMO (MULTISeq) pre-sort label.

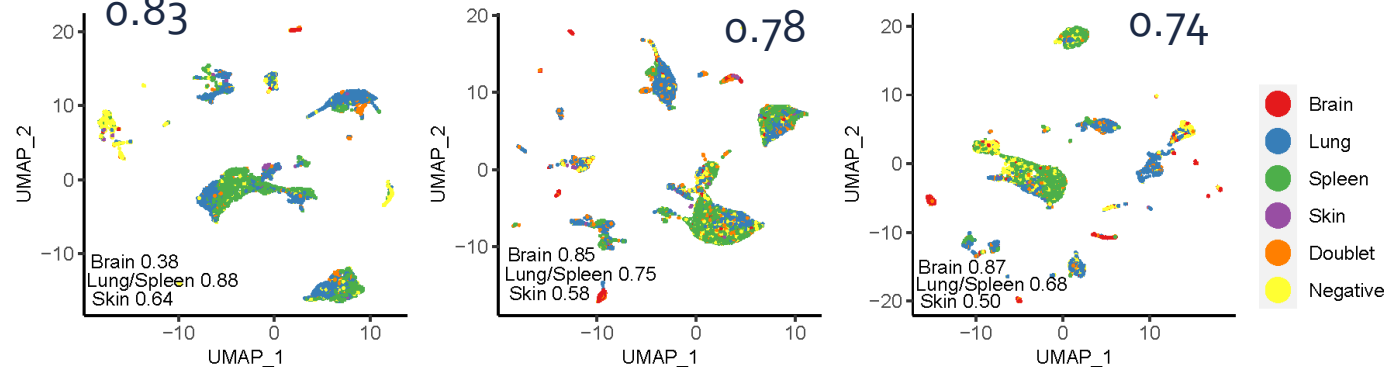
LMO (custom) pre-sort label.



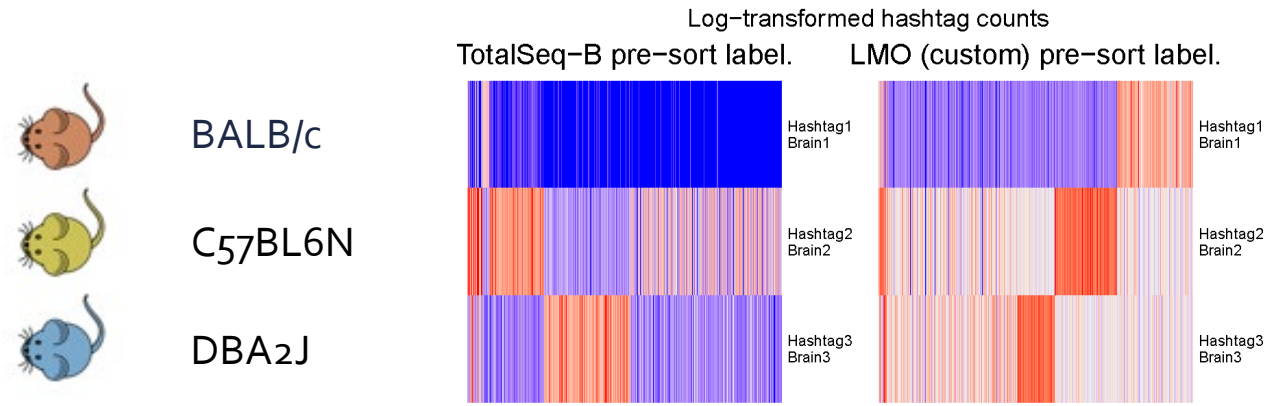
Freemuxlet annotation



MULTISeqDemux annotation and classification accuracy



Mice brain cell hashing



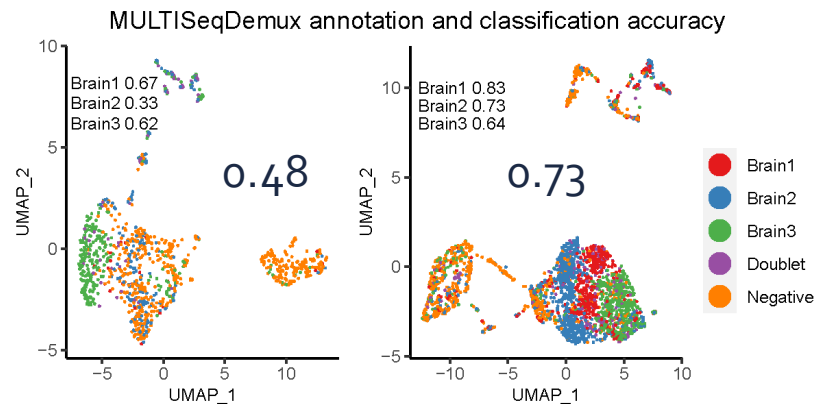
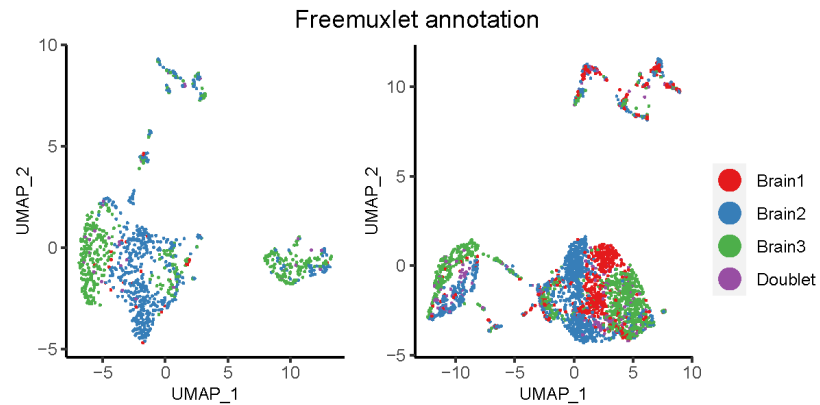
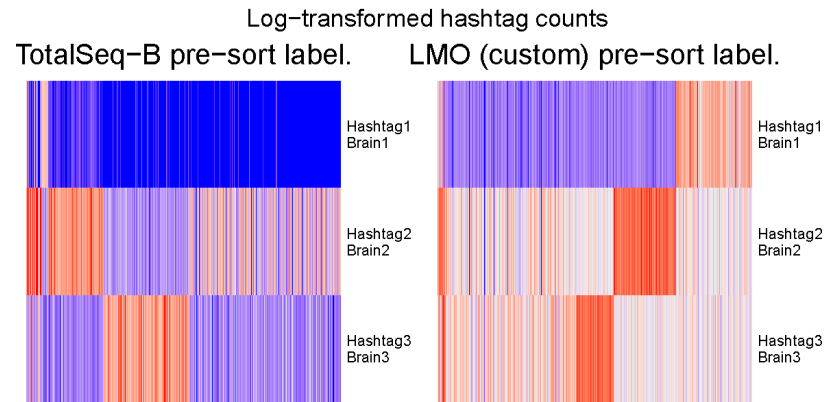
Mice brain cell hashing



BALB/c

C57BL6N

DBA2J



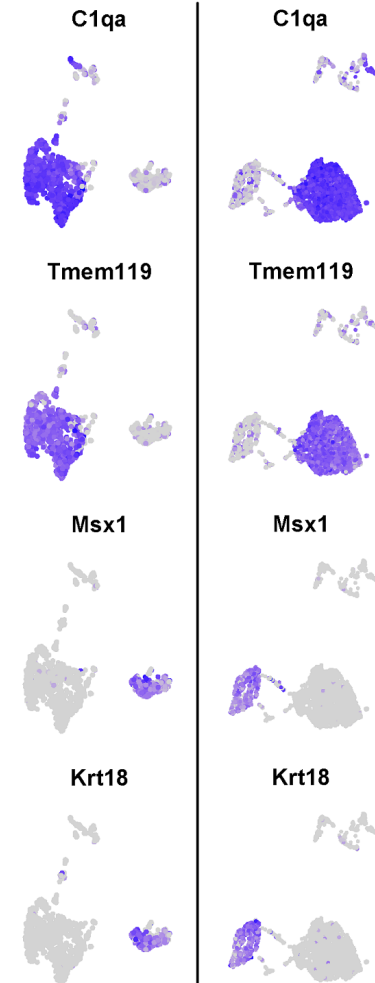
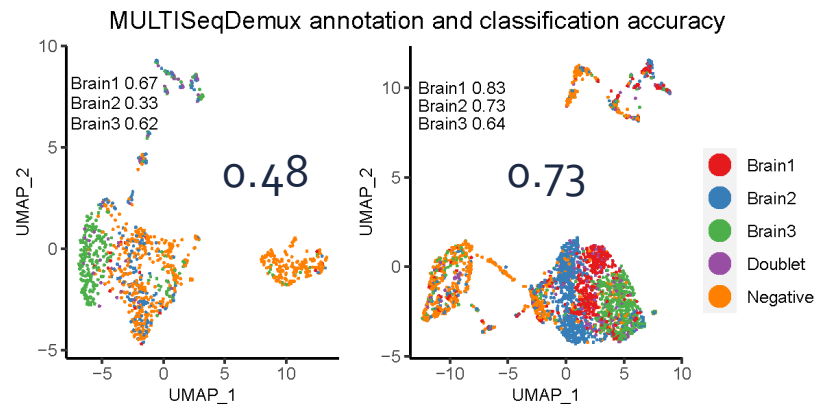
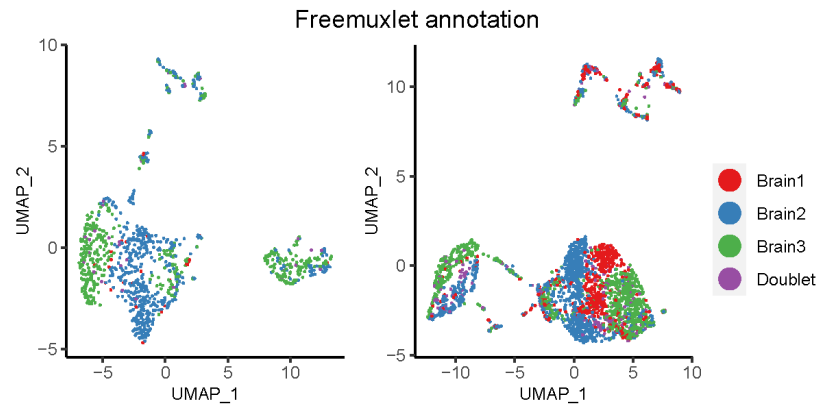
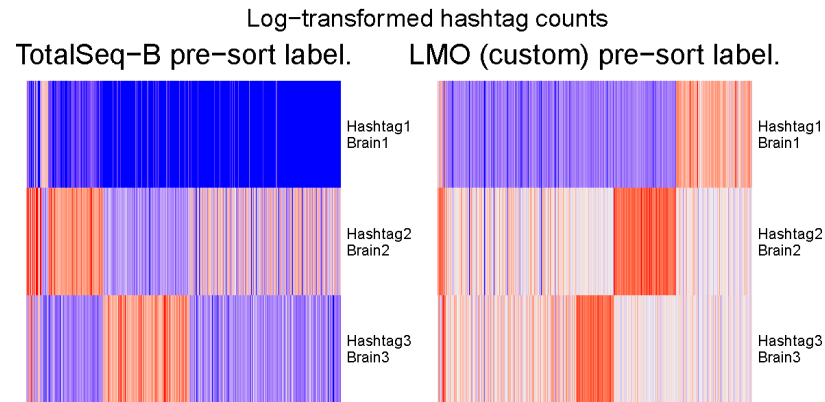
Mice brain cell hashing



BALB/c

C57BL6N

DBA2J



Comparison of demultiplexing functions

0.96	0.94	0.96	TotalSeq-A cells
0.91	0.84	0.91	TotalSeq-A cells rep2
0.96	0.94	0.96	TotalSeq-C cells
0.85	0.81	0.84	LMO (MULTI-seq) cells
0.69	0.57	0.68	LMO (custom) cells
0.76	0.74	0.84	CMO nuclei
0.43	0.54	0.51	TotalSeq-A nuclei
0.65	0.62	0.50	TotalSeq-A nuclei rep2
0.77	0.47	0.82	TotalSeq-A PBMC (healthy)
0.80	0.35	0.84	TotalSeq-A PBMC (SARS-CoV-2)
0.95	0.94	0.96	TotalSeq-B PBMC pre-sort label.
0.93	0.83	0.93	CellPlex PBMC pre-sort label.
0.52	0.85	0.83	TotalSeq-B 4 tissues pre-sort labelling
0.78	0.75	0.78	LMO (MULTISeq) 4 tissues pre-sort labelling
0.42	0.69	0.74	LMO (custom) 4 tissues pre-sort labelling
0.62	0.61	0.48	TotalSeq-B 3 tissues pre-sort labelling
0.65	0.65	0.67	TotalSeq-B 3 tissues post-sort labelling
0.76	0.77	0.48	TotalSeq-B brain pre-sort labelling
0.48	0.56	0.73	LMO (custom) brain pre-sort labelling
0.60	0.57	0.64	Average
GMMDemux	HTODemux	MULTISeqDemux	



Mylka et al., Genome Biology, 2022

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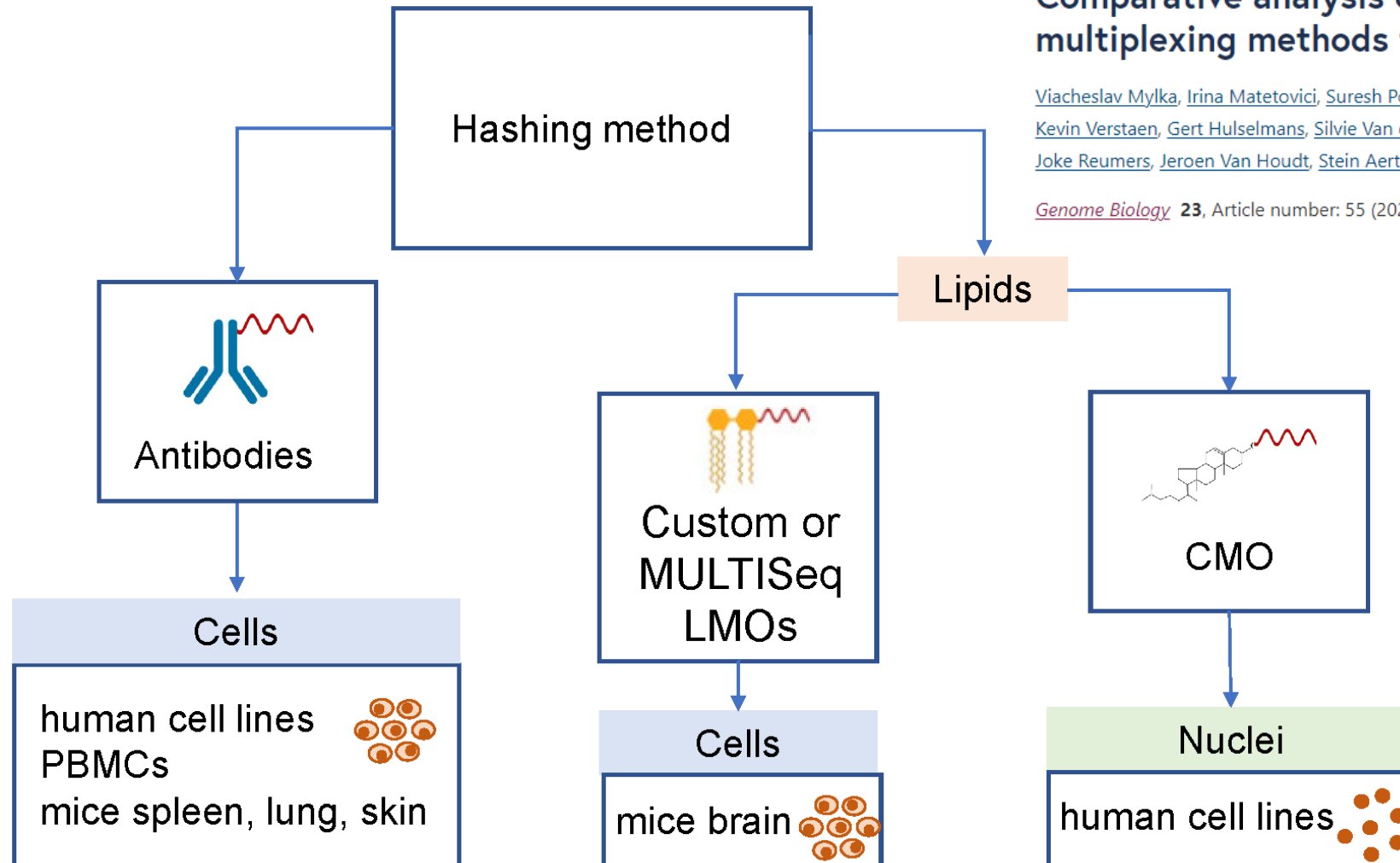
Hashing strategies: which one to use?

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](#)



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



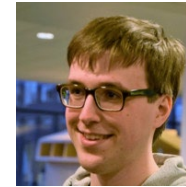
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Kia Movahedi



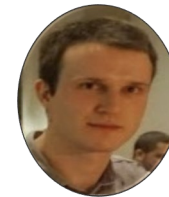
Isabelle Scheyltjens



Stein Aerts



Suresh Poovathingal



Gert Hulselmans



Viacheslav Mylka



Irina Matetovici



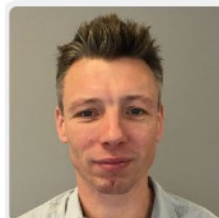
Jeroen Aerts



Silvie Van Den Hoecke



Gert Van Isterdael



Jeroen Van Houdt



Hans Wils



Joke Reumers

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Thank you!