Benchmarking single-cell and single-nucleus RNA-seq technologies

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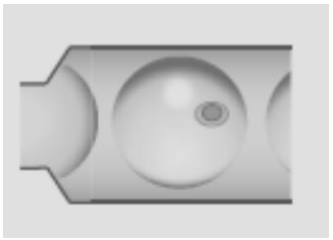
VIB's Single Cell Accelerator partnered with Janssen Pharmaceutica NV to combine their expertise and allow rapid and shared adoption of newly emerging single cell technologies. Within the collaboration we focus on key areas of the single cell field with the goal of developing an accessible single-cell technology platform for scientists.

- Benchmarking scRNA-seq technologies
- Hashing
- Single nuclei RNA-seq
- CITE-Seq

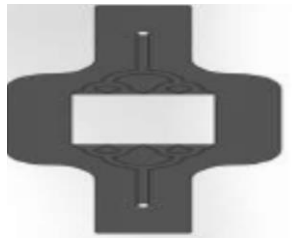
How do single-cell RNA sequencing technologies differ in expression quantification performance?

Benchmarking single-cell RNA-seq technologies



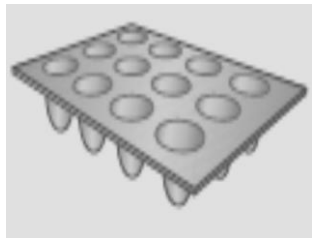
Droplet

10x Genomics
InDrop (Custom, 1CellBio)
Drop-seq (Nadia)



Slide

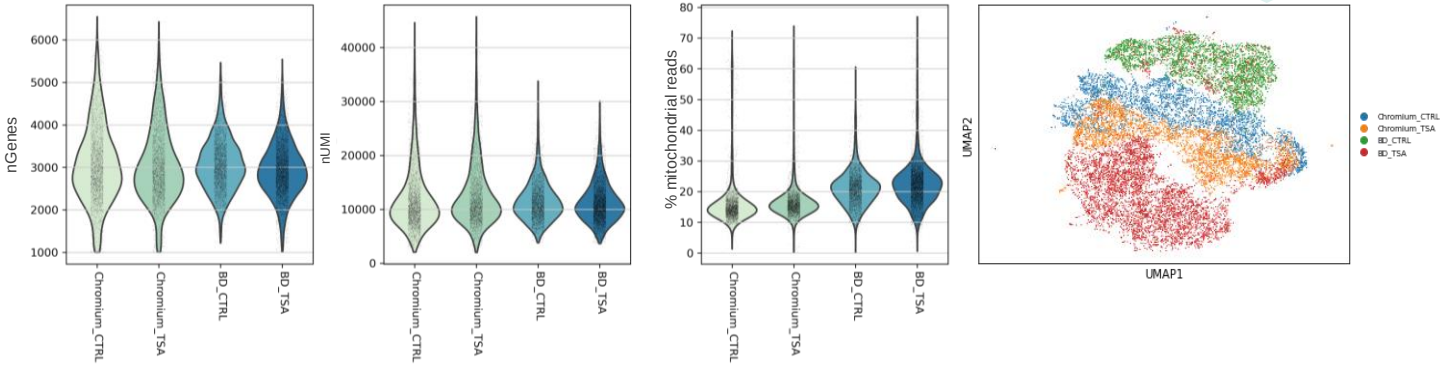
BD Rhapsody
Celsee

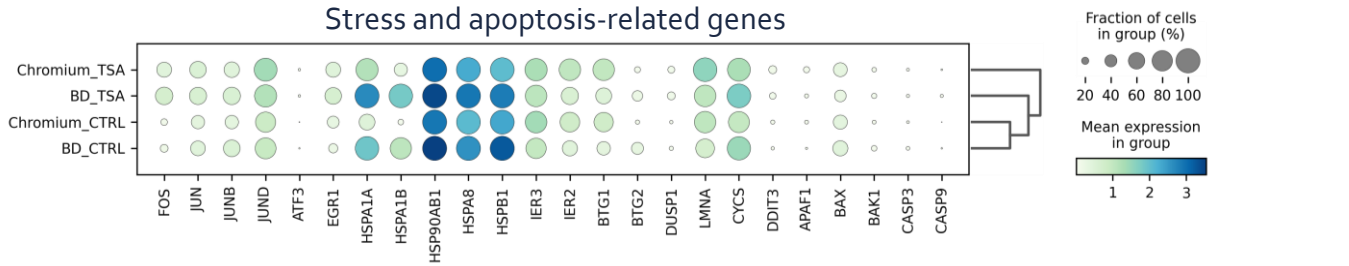


Plate

SMART-seq2
CEL-seq2

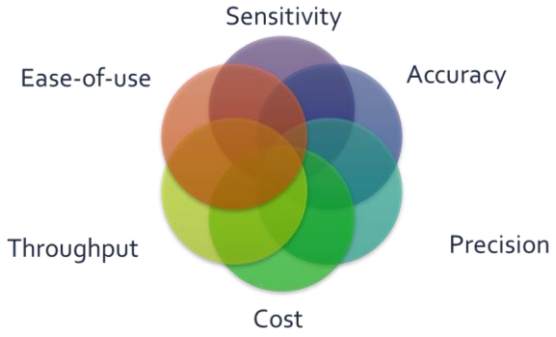
10X Chromium vs BD Rhapsody



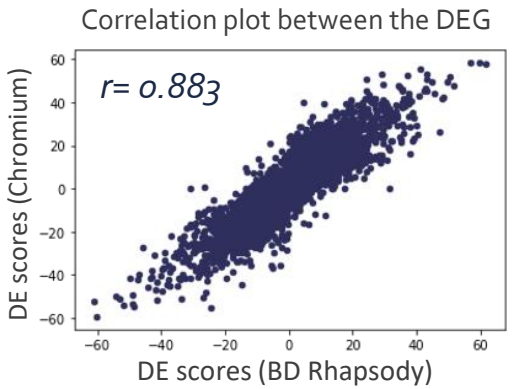


Cells (MCF7/PC3)

Control TSA



Conclusion: Each platform has advantages depending on the single cell capture mechanism and underlying chemistry.

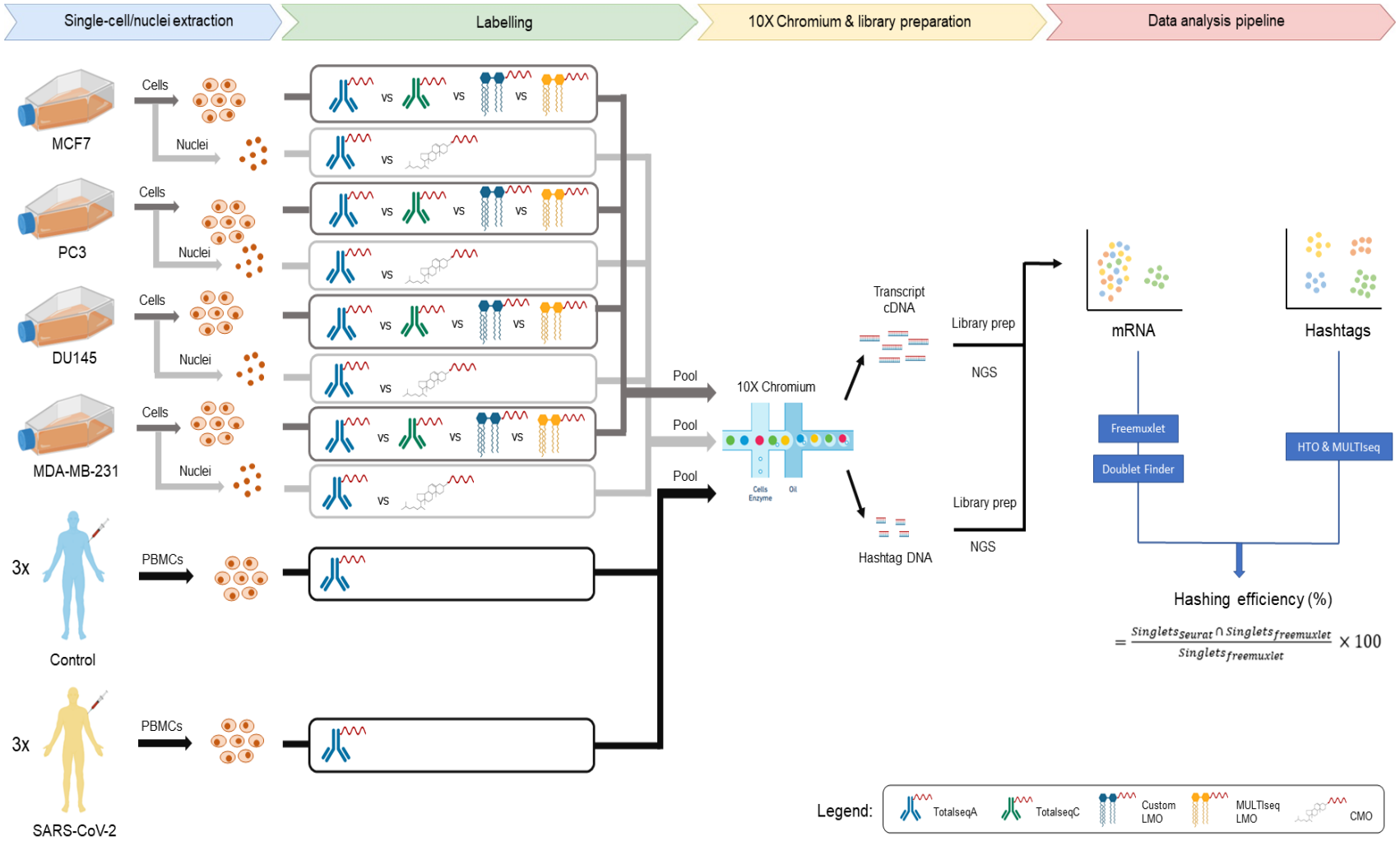


Conclusions

	Chromium v3	BD Rhapsody
Capture rate (%)	66,9	67
nGenes/Cell	~3000	~3000
mean % mito reads	13	20
multiplet cells	450	190
multiplexing	Yes	Yes
TSA signature	Yes	Yes

Comparative analysis of antibody- and lipid-based multiplexing methods for single-cell RNA-seq (Mylka et al., 2020, bioRxiv)

Hashing

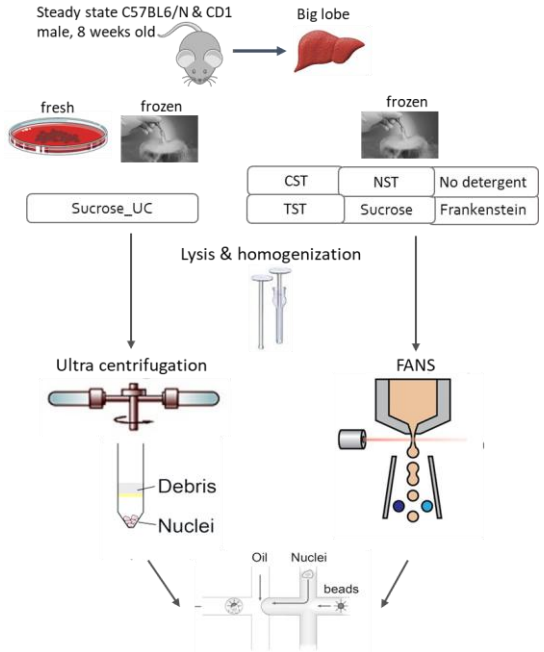


Experiment	Hashing efficiency	SD
1. TotalSeq-A cells	**95.4%**	± 0.4
2. TotalSeq-A cells rep2	**90.9%**	± 1.8
3. TotalSeq-C cells	**96.2%**	± 0.9
4. LMO (MULTI-seq) cells	**84.9%**	± 8.8
5. LMO (custom) cells	**68.5%**	± 17.4
6. CMO nuclei	**84.1%**	± 1.9
7. TotalSeq-A nuclei	**50.2%**	± 31.2
8. TotalSeq-A nuclei rep2	**63.3%**	± 30.7
9. TotalSeq-A PBMC1 (healthy)	**84.1%**	± 1.7
10. TotalSeq-A PBMC2 (SARS-CoV-2)	**83.6%**	± 2.2

Conclusions

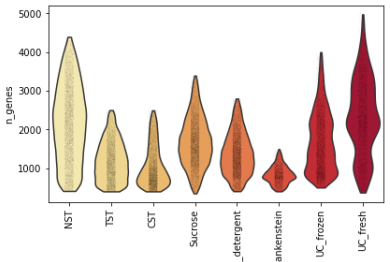
- Cells (human cell lines) -> antibody-based hashing methods appear to be superior as compared to lipid- based approaches.
- Nuclei (human cell lines) -> cholesterol-based hashing (CMO) outperforms antibody-based (TotalSeq -A) labelling.
- PBMCs -> high hashing efficiency (TotalSeq – A) for heathy and SARS-CoV-2 samples.

Protocols evaluation for nuclei isolation

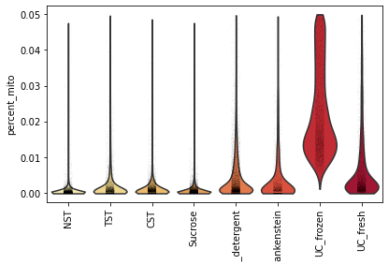


Single nuclei RNA-seq

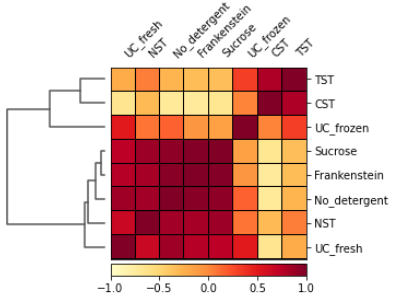
Gene number/cell



% mitochondrial reads



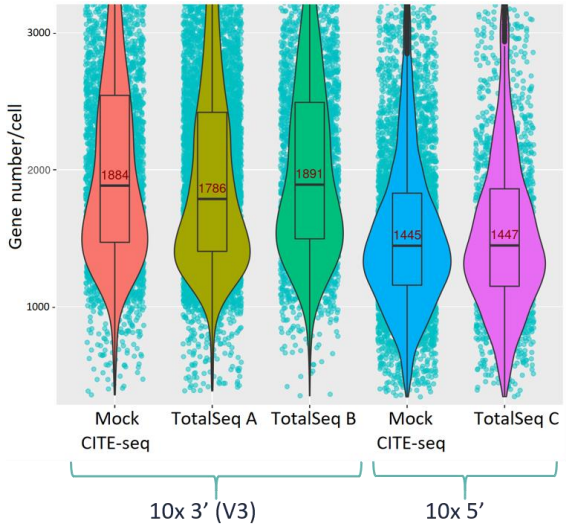
Correlation matrix (Pearson)



Conclusions

- High variations in the number of nuclei captured/protocol.
- Lower % of ambient RNA in the frozen samples purified by FANS.
- NST protocol: highest median genes per nucleus (frozen samples).
- Variations in the cell types identified/protocol.

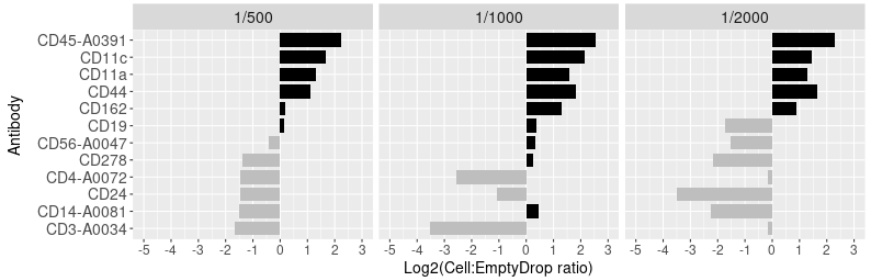
TotalSeq A/B/C antibody and CITE-seq protocol comparisons



Gene number per cell in mock CITE-seq samples (no antibodies) and 3 CITE-seq samples (31 TotalSeq A, B or C antibodies). Median values are in red. Antibody UMIs per cell are normalized across the samples.

CITE-seq Human PBMCs

TotalSeq antibody dilution comparison



Sum of the antibody-derived UMIs in cells versus empty droplets across 3 antibody dilutions. Cell number is normalized across the dilutions. An empty droplet is a droplet with less than 500 gene expression UMI.

Conclusions

- > 50% of antibody UMIs (276 TotalSeq A abs) found in empty droplets.
- Minor differences between the tested dilutions of signal-to-noise ratios.
- All 3 TotalSeq types detect major PBMC markers, with no (TotalSeq B and C) or a minor effect (TotalSeq A) on the transcriptome (31 antibodies).