Miscellaneous

Types of data / special analyses

- Multiplexed single-cell RNAseq
- Cite-seq (called also Total-seq)
- VDJ single-cell RNAseq
- VDJ 5' multiplexed single-cell RNAseq
- Spatial transcriptomics (not yet single-cell, but soon!?)
- Multi-omics
- Single nuclei RNA-seq

Multiplexed single-cell RNA-seq

- Reduce the cost of the sequencing by using a labelling for each sample then pool different samples and then sequence.
- For the analysis, the only difference compared to standard single-cell RNA-seq data is in the cellranger part.
- Key advantages of Cell Multiplexing include:
- Increased sample throughput in a single experiment
- Increased number of cells assayed in a single experiment
- Increased number of possible replicates in a single experiment
- Detection of multiplets and their removal prior to analysis

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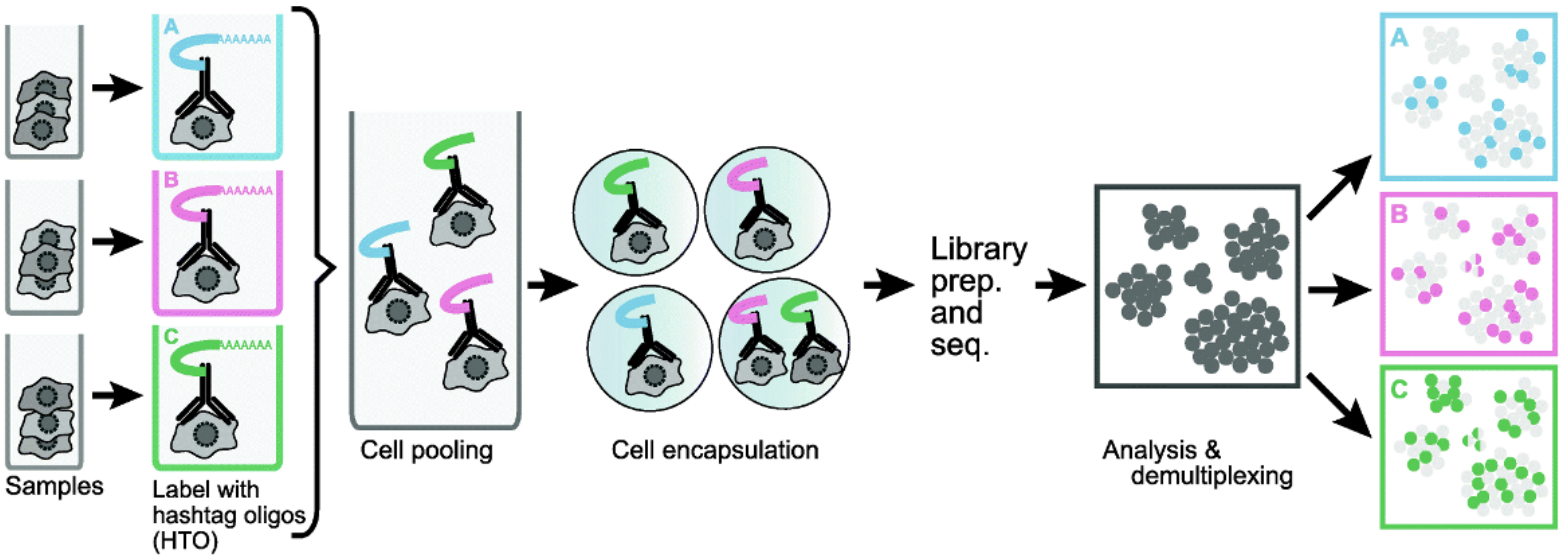
Multiplexed single-cell RNA-seq-How it works

- Cell multiplexing oligos (sometimes called hashtag oligos) or CMO are added to the cells, one CMO per sample in a pool (this means the same CMO can be used in several different pools for different samples, and this is commonly used).
- The technique is similar to measuring cell surface proteins (that we will see with the total-seq and cite-seq methods).

Multiplexed single-cell RNA-seq-How it works



Stoeckius M et al. Cell Hashing with barcoded antibodies enables multiplexing and doublet detection for single cell genomics. Genome Biol. Genome Biology; 2018;19:1–12.

cellranger multi --id Mysample345 --csv PATH_TO/Multi_file.csv

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CSV file should contain

- The [gene-expression] section has two columns that specify parameters relevant to analysis of gene expression data, such as reference genome and cell-calling parameters, as well as other all-purpose parameters.
- The [libraries] section has three required columns that specify where the input FASTQ files may be found.
- The [samples] section has two required columns that specify sample information for Cell Multiplexing.

```
[gene-expression]
reference,/path/to/transcriptome

[libraries]
fastq_id,fastqs,feature_types
gex1,/path/to/fastqs,Gene Expression
mux1,/path/to/fastqs,Multiplexing Capture

[samples]
sample_id,cmo_ids
sample1,CMO301
sample2,CMO303
```

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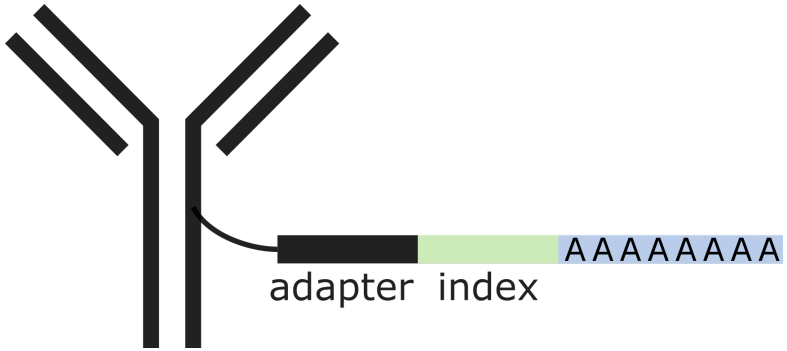
Total-seq / Cite-seq - REMINDER



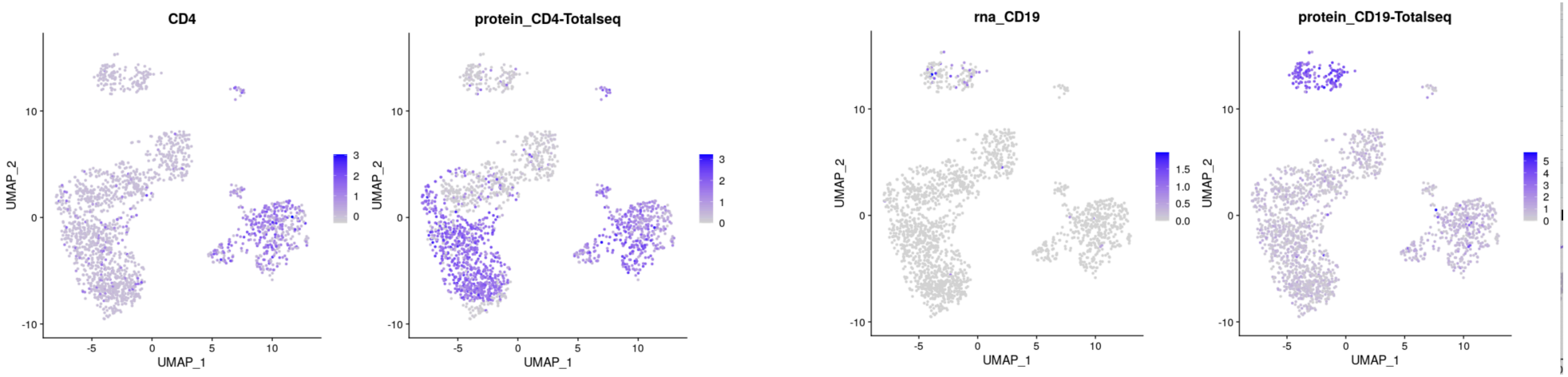
- Quantification of (cell-surface) proteins
- Together with transcriptome

Stoeckius M et al. Simultaneous epitope and transcriptome measurement in single cells. Nat Methods. 2017;14:865–8.

Total-seq / Cite-seq

- A little bit similar than multiplexing in terms of the file structure.
- This time each cell has a panel of surface proteins (or proteins) that are measured on top of the RNA-seq of each cell.
- This enables more precise annotation of cells, due to the high amount of dropouts in the RNA-seq part.

Courtesy Sarah Cattin



Total-seq cellranger

- **cellranger count --id=sample345 --libraries=library.csv --transcriptome=/path/refdata-gex-GRCh38-2020-A --feature-ref=feature_ref.csv**

library.csv file looks like this:

fastqs	sample	library_type
/opt/foo/	GEX_sample2	Gene Expression
/opt/foo/	Ab_sample2	Antibody Capture

… and feature_ref.csv like this

read	pattern
R2	5PNNNNNNNNNN(BC)

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VDJ single-cell RNAseq

- **cellranger vdj --id=sample345 –fastq=PATH_TO_FASTQ --libraries=library.csv --transcriptome=/path/refdata-cellranger-vdj-GRCh38-alts-ensembl-7.0.0 --sample=sample-name**

VDJ 5' multiplexed single-cell RNAseq

- That is where the fun starts.
- Cellranger does not allow 5' multiplexed data. This is however exactly what you can do when you want to do VDJ analysis for lower cost.
- A "not too complicated" procedure is explained in this https://www.10xgenomics.com/resources/analysis-guides/demultiplexing-and-analyzing-5%E2%80%99-immune-profiling-libraries-pooled-with-hashtags article from the 10x genomics support this is for cellranger version 7 only.
- One demultiplexes using **cellranger multi** the single-cell RNAseq part (without touching the multiplexed VDJ fastqs). This generates mapped bam files for each sample.
- These bam files are returned to fastq files (bamtofastq) by making sure that only one fastq file is created.

The csv file will look like that

[gene-expression]
reference-path,/ref/refdata-gex-mm10-2020-A/
cmo-set,/data/CellRanger/feature_reference_W1.csv
[libraries]
fastq_id,fastqs,feature_types
W1_GEX,/data/fastq/nvid00011,Gene Expression
W1_FB,/data/fastq/nvid00019,Multiplexing Capture
[samples]
sample_id,cmo_ids
24_1,A0301
18_c,A0302
12_3,A0303
6_1,A0304

VDJ 5' multiplexed single-cell RNAseq

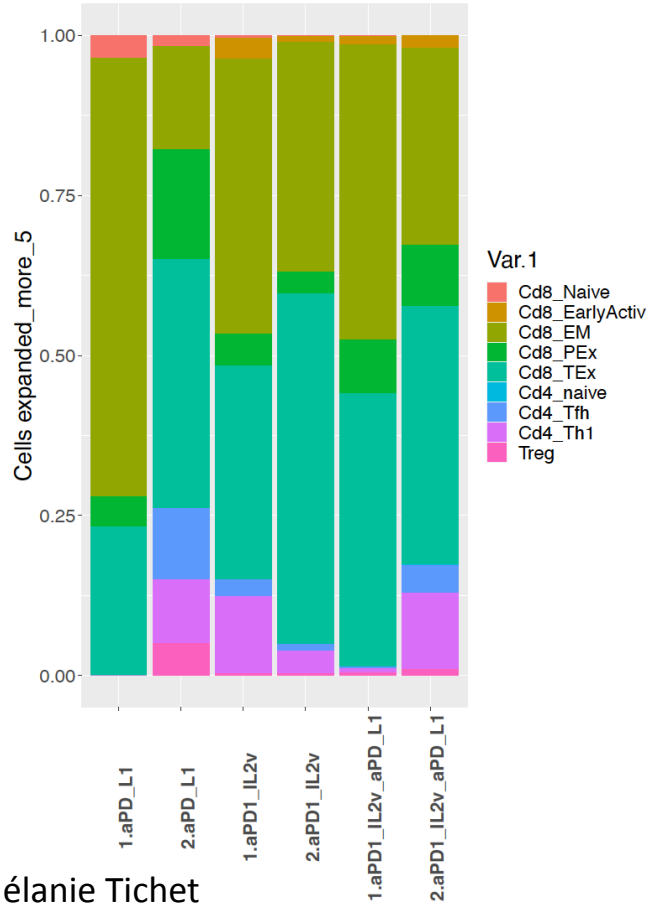
- Then the "per sample" single-cell RNAseq files are mapped back to the genome this time using the multi function with one part for the VDJ and the other for the RNAseq.
- You then obtain VDJ and RNAseq results per sample as desired.

The csv file will look like that

[gene-expression]
reference-path,/ref/refdata-gex-mm10-2020-A/
force-cells,5000
chemistry, SC5P-R2
[vdj]
reference-path,/results/refdata-cellranger-vdj-GRCm38-alts-ensembl-5.0.0
[libraries]
fastq_id,fastqs,feature_types
bamtofastq,/results/FASTQ_per_sample/24_c/W4_GEX_0_1_HNC3HDRXY/,Gene Expression
W4_VDJ,/data/fastq/nvid00018/,VDJ

The things you can do with this

- Understand the composition, expansion and diversity of the clones in your samples.
- Get the differences in T cell composition of the expanded clones (are they naïve, memory or active t-cells?)

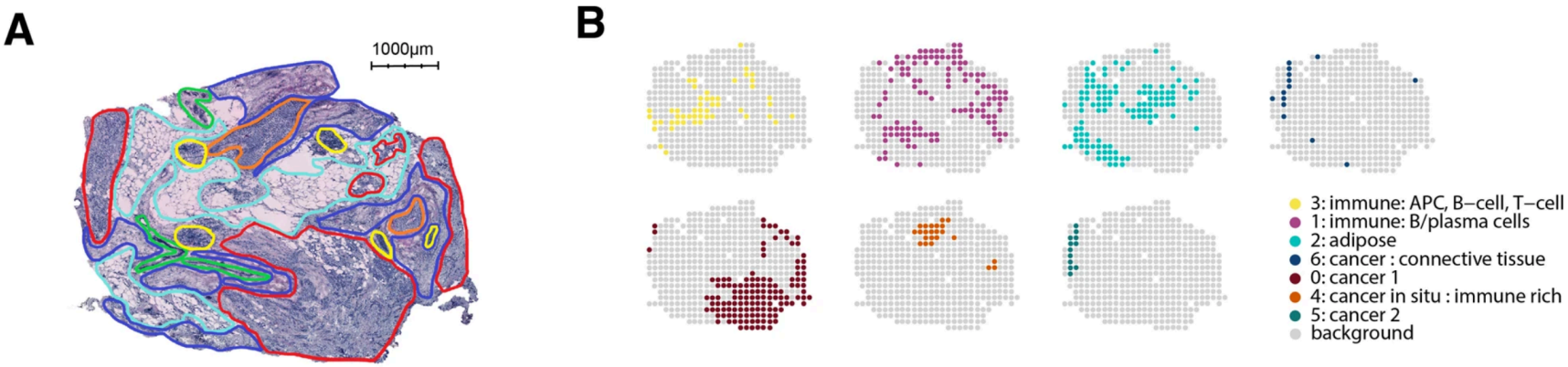


Courtesy Mélanie Tichet

Spatial transcriptomics

- On 10 micro-meter thick tissue slides you perform on spots of 55 micro-meter barcode and UMI libraries.
- This enables you to look at the resolution of 55 micro-meters the expression of cells in that spot.
- Instead of cellranger one uses for example spaceranger.
- The output files are very similar than the ones from cellranger. Except that you have another folder called spatial, with a txt file of spot and x-y coordinates of the section.
- Right now a lot of questions are still to be solved as it is not a single-cell resolution (how many cells are in one spots, how to normalise the data for example)
- Visium 10X announced that by the end of this year they will go down to 10 micro-meters of spots which then goes to a single-cell resolution.
- You can use Seurat for the analysis but also SpatialExperiment object or SingleCellExperiment object to deal with that data type.

Spatial transcriptomics example



Spatial deconvolution of HER2-positive breast cancer delineates tumor-associated cell type interactions, Alma Andersson et al. Nature 2021

spaceranger count −id −transcriptome --fastqs --sample −image −slide −area

Multi-omics

- One can combine single-cell RNA-seq with single-cell ATAC-seq, having regulome and transcriptome information on similar cells. Single cell RNA-seq then helps the ATAC-seq single cell to be annotated by doing a label transfer.
- This label transfer functions using similar techniques then the one used for integration (merging k-tables).

Single Nuclei rnaseq-Reminder

- Alternative to scRNA-seq
- For tissues difficult to dissociate
- No ribosomes -> no translation of transcription factors during processing
- Lower representation of immune cells + surface proteins
- No difference in the cellranger part, in the QC filtering of cells with high mitochondrial content is unnecessary, otherwise it is treated in the same way as single-cell RNA-seq

Denisenko E, et al. Systematic assessment of tissue dissociation and storage biases in single-cell and single-nucleus RNA-seq workflows. Genome Biol.; 2020;21:1–25.