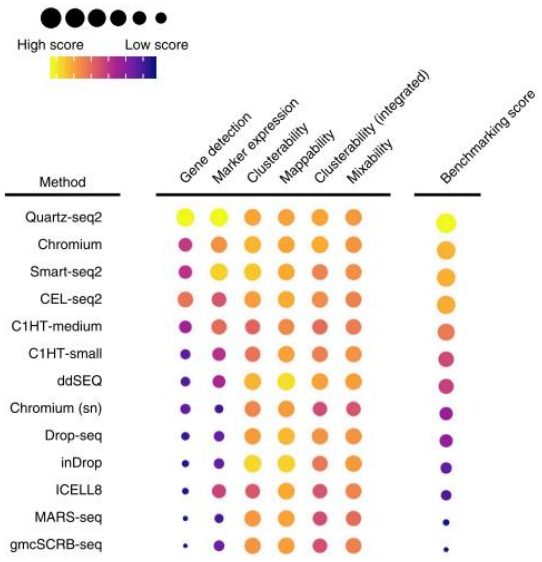
Single Cell Transcriptomics

Newsletter July 2020

Paper 1

Mereu, E *et al*. [Benchmarking single-cell RNA-sequencing protocols for cell atlas projects] *Nature Biotechnology, 2020.*

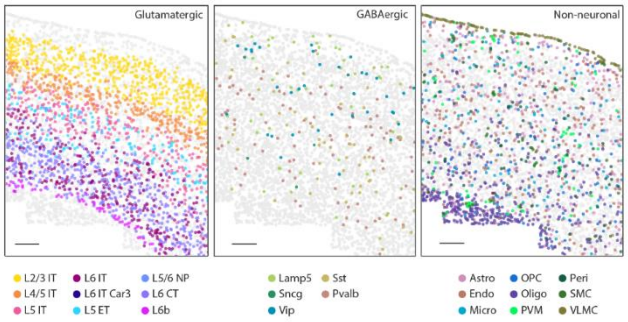
The authors performed a comprehensive comparative analysis of 13 different scRNA-seq protocols. They compared their molecule-capture efficiency by evaluating the ability of these techniques to describe tissue complexity and their suitability for creating a cell atlas. They used a heterogeneous reference sample with cells from different species to cover a broad range of cell types and states. The result of this multicenter benchmarking study provides guidance for individual as well as consortium projects to select the appropriate technique. They profiled ~3,000 cells with each scRNA-seq protocol and processed the datasets in a uniform manner. They compared the different protocols regarding efficiency of mRNA capture, library complexity, magnitude of technical biases and ability to describe different cell populations. Moreover, they assessed the integratability of datasets derived from different protocols as commonly used in consortium-driven cell atlas projects. The overall winner of this study is the plate-based technique Quartz-seq2. Its success arises probably from improved poly(A) tagging which increased the amplified cDNA yield. Besides Quartz-seq2 also Smart-seq2 showed high expression levels for marker genes. CEL-seq2 and Quartz-seq2 identified many more genes than other methods. Especially CEL-seq2 detected many weakly expressed genes outperforming all other methods.



Paper 2

Zhang, M *et al.* [Molecular, spatial and projection diversity of neurons in primary motor cortex revealed by in situ single-cell transcriptomics] *bioRxiv, 2020.*

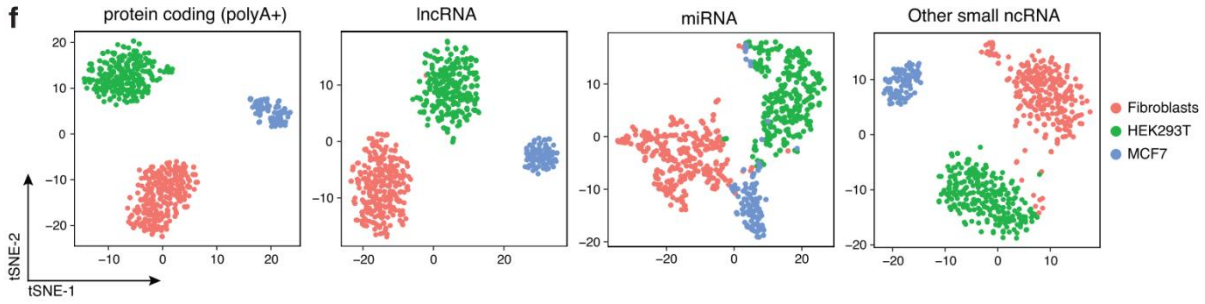
Single-cell RNA-seq allows to identify specific cell types based on their expression of marker genes and has revealed many distinct cell types in the brain. However, the sequencing-based methods do not preserve the spatial organization and connectivity of the cells, which largely contributes to our understanding of cortical circuits. The authors applied **m**ultiplexed **e**rror-**r**obust **f**luorescence **i**n **s**itu **h**ybridization (MERFISH), an in situ single-cell transcriptome-imaging method, to generate a spatial resolved cell atlas of the mouse primary cortex (MOp). They imaged and segmented ~300,000 individual cells in the MOp and identified 39 excitatory, 42 inhibitory and 14 non-neuronal cell populations. The cell census identified by MERFISH showed good correspondence to transcriptomic data derived from single-cell sequencing based method. They found that intratelencephalic (IT) cells, the largest branch of neurons in the MOp, did not form one discrete cluster but a large continuum of cells with a strong correlation between gene expression profiles of individual cells and their cortical depth positions. Moreover, they combined MERFISH with retrograde tracing experiments and showed that each neuronal cell cluster of the MOp can project to multiple regions and each region can receive input from many clusters. Thus, projections of the MOp neurons do not occur in a one-cell-type-to-one-target-region manner. To sum up, they generated a high-resolution spatial and projection map of molecularly defined cell types in the MOp. The technique they used here can be applied to create maps of a wide range of systems.



Paper 3

Isakova, A *et al.* [Single cell profiling of total RNA using Smart-seq-total] *bioRxiv, 2020.*

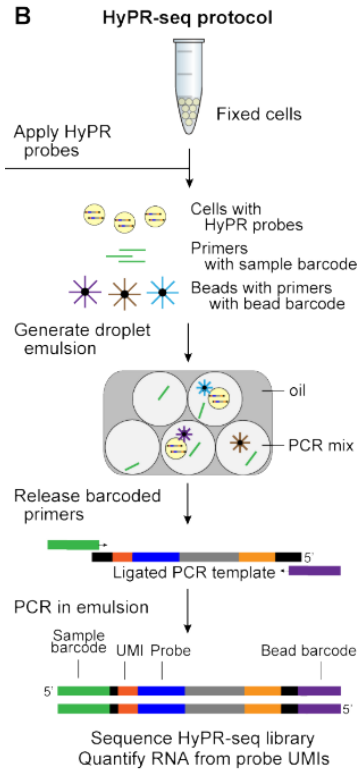
Until now it has not been possible to simultaneously assess all RNA types within a cell. Most effort has been put into single-cell RNA-seq methods focused on protein-coding RNA. Some groups have shown techniques to measure non-coding RNA in single cells, however these approaches only target a subset of non-coding RNAs which are either long (e.g. lncRNA or circRNA) or short (e.g. miRNA). Smart-seq-total is a scalable method designed to capture both coding and non-coding transcripts regardless of their length. This allows to map the regulatory connection between coding, and different types of non-coding transcripts within a cell. The specialty of the Smart-seq-total technique compared to similar approaches is that it uses an optimized template switch oligo (TSO) which can be rapidly eliminated from the reaction directly after the reverse transcription. Furthermore, a CRISPR-mediated removal of highly abundant ribosomal RNA from the final library is performed. They applied the protocol to 3 different human cell lines. They detected a broad spectrum of transcripts such as mRNA, miRNA, lncRNA, and snoRNA in each profiled cell and were able to distinguish the 3 different cell types based on the abundance of non-coding RNA alone. Moreover, they could show that the abundance of certain non-coding transcripts changed during the cell-cycle as well as during developmental stages.



Paper 4

Marshall, JL *et al.* [HyPR-seq: Single-cell quantification of chosen RNAs via hybridization and sequencing of DNA probes] *bioRxiv, 2020.*

Hybridization of Probes to RNA for sequencing (HyPR-seq) is a powerful method for targeted RNA profiling in single cells. It allows to sensitively quantify the expression of up to 100 chosen genes including low-abundance and non-polyadenylated transcripts in more than 100,000 single cells. By directing the sequencing power to the transcripts of interest HyPR-seq reduces costs extremely compared to whole-transcriptome scRNA-seq. HyPR-seq builds up on single molecule fluorescence *in situ* hybridization (smFISH) were labeled single-stranded DNA (ssDNA) probes hybridize to the transcripts of interest. Instead of using imaging techniques as readout, HyPR-seq uses next-generation DNA sequencing to enable simultaneous readouts of hundreds of probes in thousands of single cells. They showed that HyPR-seq can profile the effects of CRISPR perturbations on individual genes of interest, detect time-resolved changes in gene expression via measurements of gene introns, which are non-polyadenylated RNA, and detect low-abundance marker genes of rare cell types in complex tissues. However, there are several limitations of the HyPR-seq technique. In this method the RNA molecule is not sequenced itself making it unsuitable for deteccitng RNA sequence modifications. Due to several washes, which result in cell loss, the protocol requires to start with 1 million or more cells. Finally, since HyPR-seq involves hybridization of probes, it can be difficult to specifically target RNA transcripts that have a high sequence homology with other transcripts.



Next Single Cell Seminar

Next seminar will take place in August.

If you would like to announce anything single cell related, being it job announcement, event, your published paper, technology development etc, please contact us.

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