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Review article

Single-cell and spatial transcriptomics: deciphering brain complexity in health and disease

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Supplementary Information

Obtaining single cells and nuclei from CNS

Single-cell and -nuclei suspensions are obtained from CNS by application of protocols involving mild enzymatic dissociation of the tissue, washing steps, debris removal and counting of live cells/nuclei for subsequent encapsulation of specific number of cells/nuclei. These protocols require rapid handling to maintain RNA integrity and minimize cell death.

Single-cell suspension from CNS tissues. Live cells are obtained by enzymatic and mechanical dissociation of CNS tissues and usually optimized according to the source of the input martial (e.g. brain region of choice). A critical step are monitoring cell recovery rates and cell's viability. Obtaining intact cells from mouse embryo and early postnatal brains is typically done by papain digestion, followed by mild mechanical trituration and removal of debris by gradient centrifugation or FACS sorting. These protocols allow the recovery of both glia and neurons. As for obtaining live cells from adult mouse brain, protocols based on acute slice preparation followed by mild enzymatic dissociation have been published and the duration of the digestion is optimized for each brain region separately[39,40]. Similar approaches are used for the human embryo brain to achieve representative cell numbers and glia to neuron ratios. Human brain organoids and patient tumor samples are also amenable to dissociation resulting in good quality of live single cell suspension, while adult human clinical samples are more often subjected to single-nucleus RNA seq (the samples are usually cryopreserved and the isolation of live intact cells is hampered).

Single-nuclei suspension. Single-nuclei preparation can be performed from fresh tissue material as well as from snap-frozen tissue, or otherwise preserved material (e.g. in RNAlater). The procedures are typically adjusted to tissue of origin when it comes to the composition of lysis buffers and duration of certain steps[20], and involve mild lysis and homogenization of the tissue (Dounce tissue grinder or chopping), followed by nuclei wash, removal of the tissue debris and nuclei resuspension. Optiprep or sucrose-gradient ultracentrifugation can be used for cleaner separation of nuclei from the remaining tissue lysate. Minimizing the presence of nuclear aggregates, cytoplasmic nucleic acids, and potential inhibitors of reverse transcription are critical to obtaining high quality data. To meet these requirements, nuclei after resuspension can be stained with DAPI and FACS-sorted.

Slicing tissues for Spatial Transcriptomics. Standard methods of tissue sectioning are applied for preparing material for spatial transcriptomics protocols. Formalin-fixed paraffin embedded (FFPE) or fresh frozen tissue sections can be accustomed. 5 micron or 10 micron-thick sections are used in the Nanostring GeoMX digital spatial profiling protocols and Visium (10x Genomics) platform, respectively. Gene expression signal is limited to the diameter of barcoded spots e.g. 55 µm spots in Visium enable capturing of 1 to 10 cells per one spot, typically measuring hundreds to thousands of genes in that area. For example, Maynard et al. applied Visium ST to human dorsolateral prefrontal cortex (DLPF) sections, and obtained data from all layers of DLPF with means of >3,400 transcripts and of >1,700 genes per spot. Similar approaches have achieved a higher resolution of 10 µm (Slide-seq24), and 2 µm (HDST, high-definition spatial transcriptomics), respectively. Nanostring GeoMX takes advantage of multiple primary antibodies conjugated to indexing DNA oligos with a UV photocleavable

linker. The sensitivity can account for ~1000 genes for 50 µm region, but also depends on the number of targeted transcripts per assay (can be customized or off-the-shelf 'whole transcriptome atlas' assay covers ~18,000 protein-coding human genes)[32].