Cell nuclei preparation

A single-cell nuclei suspension was prepared from FFPE tissues using the SeekMate FFPE Dissociation Kit (SeekGene K02301-0801). Briefly, 50μm scrolls from the FFPE block were deparaffinized, rehydrated, and homogenized in pre-cold lysis buffer using a Dounce homogenizer(Sigma Aldrich D8938). After washing, the number of nuclei was estimated using SeekMate Tinitan Fluorescence Cell Counter (SeekGene M002C) with AO/PI reagent. Subsequently, nuclear RNA was extracted using the SeekOne® DD Single Cell Decrosslinking Kit (Seekgene K02101-0806), and its integrity was analyzed on Agilent 4200 TapeStation system (Agilent G2991BA). Qualified nuclei were pre-decrosslinked and re-fixed with the SeekMate pre-Decrosslinking Kit (SeekMate K02301-0802) according to the manufacturer's protocols. The treated nuclei were then recounted and adjusted to a concentration of 3,000~4,000 nuclei/μl before being placed on ice for further use.

Single-cell RNA-seq library construction and sequencing

Single-cell RNA-Seq libraries were prepared using SeekOne™ DD Single Cell MPLSeq Kit (SeekGene Catalog No.K03201) according to the manufacturer's instructions.In brief, a total of 40,000–70,000 nuclei were transferred to a PCR tube, and whole-transcriptome probes were also added to hybridize with their complementary target nuclear RNAs, followed by incubation at 42℃ for 120 min to complete the hybridization reaction. After hybridization, the nuclei were washed three times with 0.1% Trition X100 in PBS to remove unbound probes. Subsequently, the washed nuclei were transferred to a new reaction system for multiplex tag hybridization: multiplex ligation tags (MLTs) were added to the system to hybridize with the pre-bound probes, and the reaction was incubated at 35 °C for 15 min. To reduce background noise, unbound MLTs are removed by washing three times with 0.1% Trition X100 in PBS. An appropriate number of nuclei were mixed with ligation and extension reagents, and the mixture was loaded into the sample wells of the SeekOne® DD Chip S3 (Chip S3). Barcoded Hydrogel Beads (BHBs) and partitioning oil were then dispensed into corresponding wells separately in Chip S3. The nuclei-containing ligation/extension reagents and BHBs were encapsulated into emulsion droplets using the SeekOne® Digital Droplet System. Immediately transferring the emulsion droplets into PCR tubes, the following thermal program was executed: incubation at 30 °C for 60 min, followed extension at 60 °C for 45 min, and final heat deactivation at 80 °C for 20 min, which obtained barcoded probe products. Emulsion droplets were then broken to release the barcoded probe products, and barcoded probe products were purified to remove oil and residual reagents. After pre-PCR enrichment and purification, one-fourth volume of the barcoded probe products was amplified via index PCR, and the indexed libraries were purified using VAHTS DNA Clean Beads (Vazyme, Catalog No. N411-01). Finally, the concentration and quality of the purified libraries were assessed using a Qubit Fluorometer (Thermo Fisher Scientific, Catalog No. Q33226) and a Bio-Fragment Analyzer (Bioptic, Model Qsep400), respectively. And qualified libraries were sequenced on an Illumina NovaSeq 6000 platform with a paired-end (PE) read length of 150 bp.

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