

Tissue Preparation:

Fresh surgical specimens were immediately preserved and transported in ice-cold sCellLive™ Tissue Preservation Solution (Singleron) and proceeded to the next step as quickly as possible. Rinse the tissue 3 times with HBSS and cut the tissue into small pieces. The tissue was dissociated into pieces with sCellLive™ Tissue Dissociation Solution (37°C, 15 min) and filtered through a 40-µm sterile filter. Add GEXSCOPE® Red Blood Cell Lysis Buffer to lysed erythrocytes (5-8 minutes at room temperature) and centrifuge (300 g, 4°C, 5 minutes). The centrifuged cells were resuspended in PBS and cell viability was determined by trypan blue staining.

Sequencing procedures:

Single cell suspensions (2×10^5 cells/mL) were loaded onto a microtiter chip using the Singleron Matrix® Single Cell Processing System. Barcoded beads captured mRNA for reverse transcription and PCR amplification. The resulting cDNA was fragmented and ligated to the sequencing adapter. The scRNA-seq libraries were constructed according to the GEXSCOPE® Single Cell RNA Library Kits (Singleron) [1], individual libraries were diluted to 4 nM, merged, and sequenced on an Illumina novaseq 6000 with an end read of 150 bp.

- [1] Dura B, Choi JY, Zhang K, et al. scFTD-seq: freeze-thaw lysis based, portable approach toward highly distributed single-cell 3' mRNA profiling. *Nucleic Acids Res.* 2019 Feb 20;47(3):e16.