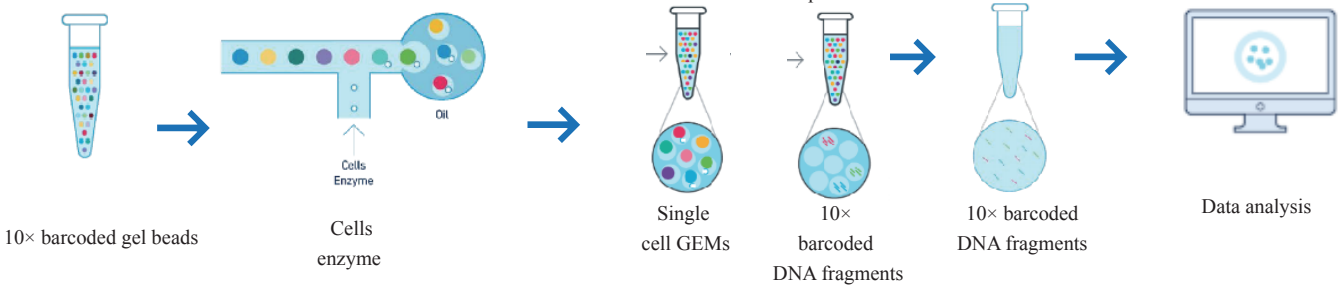
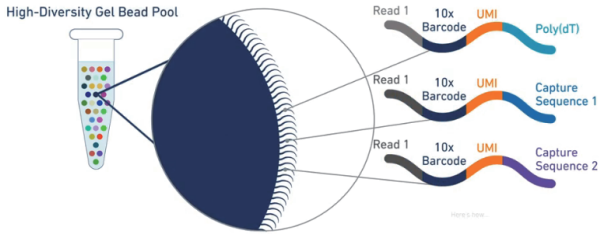
Single-cell RNA Sequencing

The advance in single cell capturing and individual library construction technique combining with high-throughput sequencing allows gene expression studies on cell-by-cell basis. It enables a deeper and complete system analysis on complex cell populations, in which it largely avoid masking of their heterogeneity by taking average of all cells, as done in bulk RNA sequencing. BMK provides 10× Genomics ChromiumTM based single-cell RNA sequencing service. This service has been widely used in studies on disease related studies, such as immune cell differentiation, tumor heterogeneity, tissue development, etc.



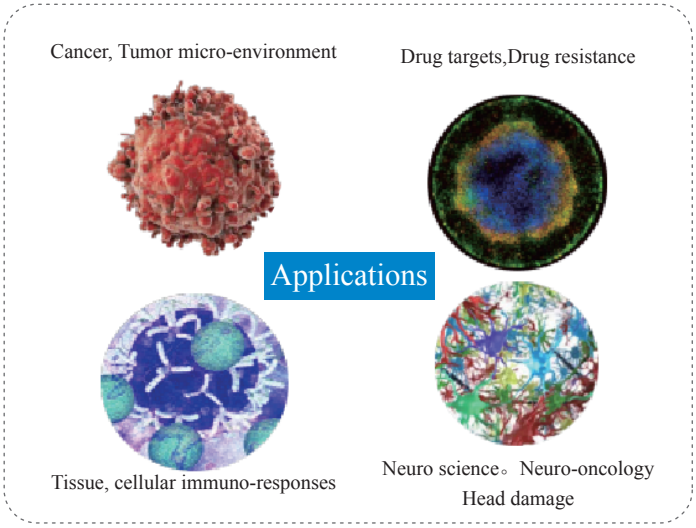
Technical work flow

The isolation of cells is achieved by 10× Genomics ChromiumTM , which consists eight-channel microfluidics system with double crossings. In this system, a gel beads with barcodes and primer, enzymes and a single cell are encapsulated in nanoliter-sized oil drop, generating Gel Bead-in-Emulsion (GEM). Once GEM are formed, cell lysis and release of barcodes are performed in each GEM. mRNA are reverse transcribed into cDNA molecules wih 10× barcodes and UMI, which are further subject to standard sequencing library construction.



Service advantages

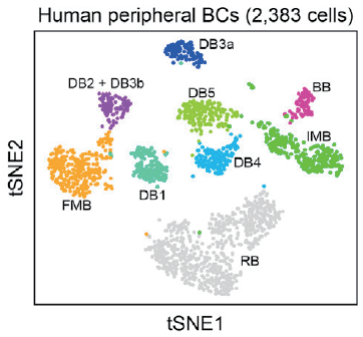
- **Highly-efficient single-cell capturing:**
  Chromium suite enables high-throughput capturing and labeling of 500 to 10,000 cells per library.
- **Diverse bioinformatics analysis:**
  In addition to basic bioinformatics, the robust bioinformtics group can also provide advanced and personalized data interpretation, such as cell differentiation trajectory.
- **Highly-experienced in library construction:**
  BMK is one of the earliest 10× Genomics related service providers. We have accumulated massive experience in single-cell library construction and reverse transcription and library construction on ultra-low RNA samples.
- **Integrated service:**
  BMK provides integrated service single-cell RNA sequencing service pack contai -ning single cell capturing, library construction, sequencing, standard bioinformatics analysis, advanced bioinformatics analysis and customized data analysis.



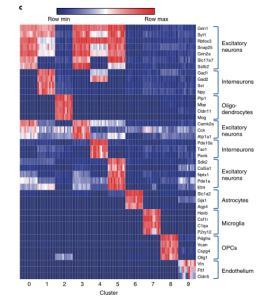
Service specifications

Library	Platform	Data volume	Sample
10× Genomics single-cell library	10× Genomics Illumina PE150	100,000 reads/cell approx. 100-200 Gb	Cell number: >2× 10^5 Cell conc. at 700-1,200 cell/μL Cell diameter: 10-40 μm Cell viability: approx. 85%

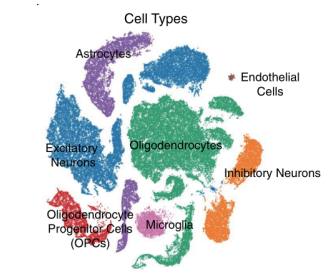
Demo results



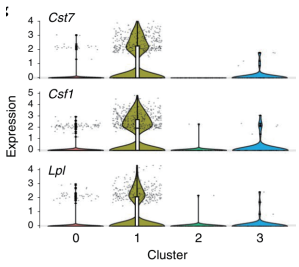
t-SNE cell clustering



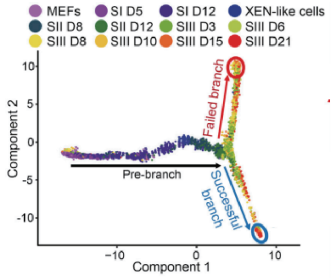
Heatmap: gene expression clustering across cell sub-populations



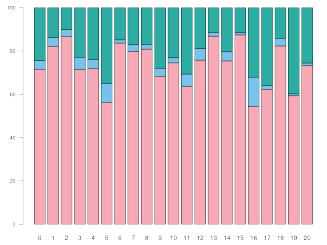
Cell sub-population identification



Gene expression distribution of specific genes



Cell trajectory analysis/pseudotime

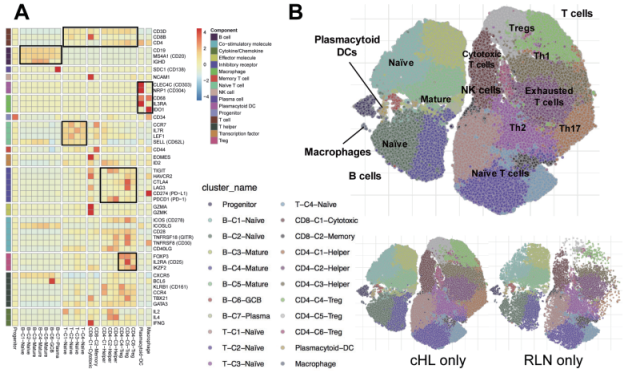


Cell cycle identification of sub-population

Case study

Title: Single-cell transcriptome analysis reveals disease defining T-cell subsets in the tumor microenvironment of classic Hodgkin Lymphoma. Cancer Discovery, IF=29.497

In this study, 22 tissue specimen of Hodgkin Lymphoma(HL) and 5 reactive lymph nodes were processed for single-cell RNA sequencing. Over 127,000 cells in total were isolated and subjected to single-cell RNA sequencing, which, for the first time, revealed HL-specific phenotypes in immuno microenvironment at single-cell resolution. Gene expression profiling at single-cell level identified a novel HL-related T-cell subset, which shown significant expression of the inhibitory receptor LAF3. Functional analyses furter confirmed LAG3+ T-cell subsets as immunosuppression mediator. Spacial assessment of LAG3+ T-cells and HRS cells shown that LAG3+ T-cells were significantly increased around MHC II-deficient tumor cells. Application of single-cell RNA sequencing in HL microenviornment study provided new insights in immune cell position in tumor microenvironment at single-cell resolution, which further suggested new biomarkers and novel ideas in treatments targeting immune checkpoints.



Immune cell atlas of the Hodgkin lymphoma(HL) microenvironment revealed by single-cell RNA sequencing

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