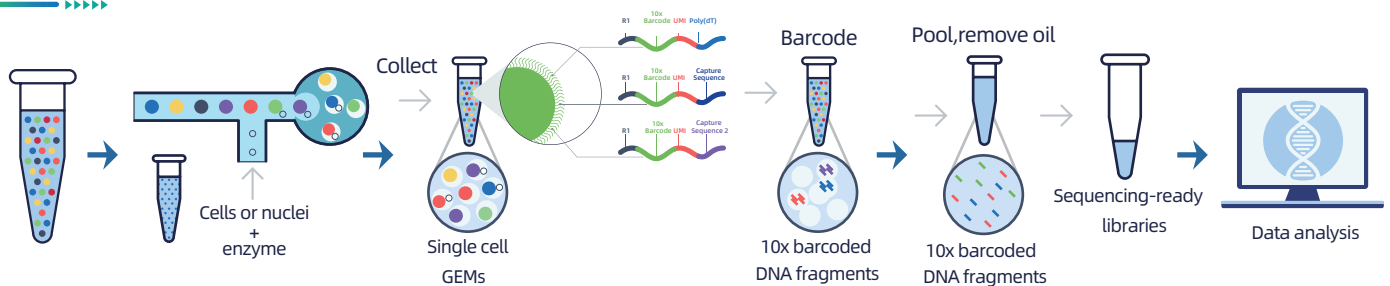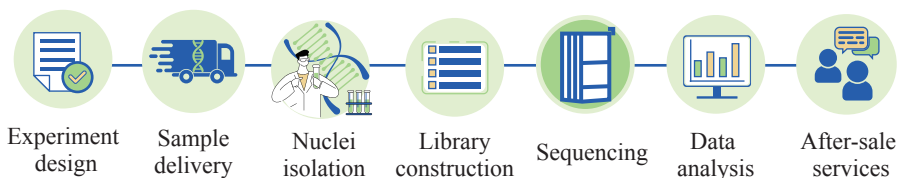# Single- nucleus RNA Sequencing

Advancements in single cell capturing and individual library construction techniques, combined with high-throughput sequencing, have revolutionized gene expression studies on a cell-by-cell basis. This approach allows for a compreh -ensive analysis of complex cell populations, avoiding the masking of heterogeneity that occurs when taking the av -erage of all cells. In cases where certain cells cannot be made into single-cell suspensions, nucleus extraction from tissues and prepared into single-nucleus suspension for single-cell sequencing is necessary. BMK offers the 10× Genomics ChromiumTM based single-cell RNA sequencing service, which is widely utilized in disease-related research, like immune cell differentiation, tumor heterogeneity, and tissue development.

Technical Workflow

Collect → Single cell GEMs → Barcode → 10x barcoded DNA fragments → Pool, remove oil → 10x barcoded DNA fragments → Sequencing-ready libraries → Data analysis

Cells or nuclei + enzyme

Service Workflow

Experiment design → Sample delivery → Nuclei isolation → Library construction → Sequencing → Data analysis → After-sale services

Bioinformation list

● Data Quality Contrl

- Raw data quality score
- Statistics on sequencing output
- Library quality control: Data saturation, Gene expression quantification
- Statistics on single nuclei counts and read counts per nuclei

● Inner / Inter sample analysis

- Gene expression matrix based cell clustering
- **Gene expression analysis:** Gene expression quantification and distribution
- **Differential expression analysis:** marker gene identification, known cell type identif -ication

- Protein interaction on marker genes of each cluster
- Hierarchical clustering on DEGs
- Prediction on TF of DEGs
- Protein interaction analysis of DEGs
- Annotation of DEGs on disease database
- Function annotation and enrichment of DEGs and gene set of each cluster

Service Advantages

Single-nucleus vs Single-cell

Single-nucleus	Single-cell
Unlimited cell diameter	Cell diameter: 10-40 μm
The material can be frozen tissue	The material must be fresh tissue
Low stress of frozen cells	Enzyme treatment may cause cell stress reaction
No red blood cells need to be removed	Red blood cells need to be removed
Nuclear expresses bioinformation	The whole cell expresses bioinformation

Tissue not suitable for single cell suspension preparation

| Cell / Tissue	Reason
Unfresh frozen tissue	Unable to get fresh or long-saved organizations
Muscle cell, Megakaryocyte, Fat…	Cell diameter is too large to enter the instrument
Liver…	Too fragile to break, unable to distinguish single cells
Neuron cell, Brain…	More sensitive, easy to stress, will change the sequencing results
Pancreas, Thyroid…	Rich in endogenous enzymes, affecting the production of single cell suspension

Service Specifications

Library	Platform	Data volume
10× Genomics single-nucleus library	10× Genomics Illumina PE150	100,000 reads/cell approx. 100-200 Gb

Sample Requirements

Cell	Tissue
Cell number: >2× 10^5 Cell conc. at 700-1,200 cell/μL	≥200 mg

Demo Results

t-SNE cell clustering
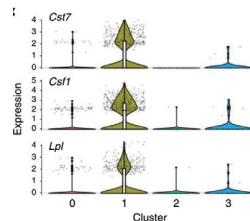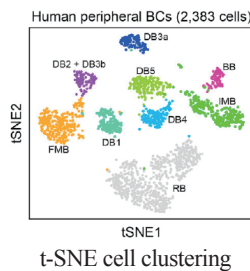
Heatmap: gene expression clustering across cell sub-populations

Cell sub-population identification

Gene expression distribution of specific genes
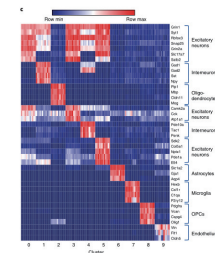
Cell trajectory analysis/pseudotime
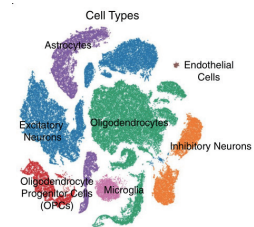
Cell cycle identification of sub-population
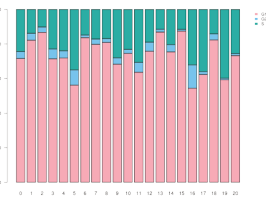
Featured Publications

Year	Journal	Title
2023	Int J Biol Sci	Integrating Spatial Transcriptomics and Single-nucleus RNA Sequencing Reveals the Potential Therapeutic Strategies for Uterine Leiomyoma
2023	Phytomedicine	Qi-Po-Sheng-Mai granule ameliorates Ach-CaCl2 -induced atrial fibrillation by regulating calcium homeostasis in cardiomyocytes

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