MedGenome Inc. Broadens Single Cell Transcriptome and Epigenome Profiling: From Tissues to single nuclei RNA (snRNA) Sequencing and Data Analysis

Abstract

Single-cell transcriptomics has revolutionized genomics and is now an integral part of therapeutics and diagnostics research. Single cell RNA sequencing (scRNA-seq) enables the analysis of gene expression at single cell resolution via droplet-based cell capture methods that rely on microfluidic instruments such as those developed by 10x genomics. While this technology is now routinely used to study a variety of research areas including the study of cellular development, the identification of cell types and states, the exploration of human disease and the development of stem cell technologies, there are several instances where it is much harder to obtain intact cells (e.g., interconnected neurons, flash frozen or cryopreserved tissues). Furthermore, the logistics and budgetary challenges of obtaining fresh tissues can often lead to significant delays for obtaining research projects. Approaches to isolate cells from such difficult sources such as flow cytometry places the cells under stress, which substantially alters gene expression. In such cases, the use of nuclei for RNA-seq avoids the difficulties involved in obtaining undamaged whole cells. To overcome this limitation, MedGenome has developed a streamlined protocol to isolate nuclei from different sources of cells (fresh and cryopreserved cells). We demonstrate that these isolated nuclei can be used for downstream applications including gene expression profiling and epigenome profiling.

Methods

Nuclei isolation requires chemical or mechanical disruption of the cytoplasmic membrane while retaining the integrity of the nuclear membrane. Any type of chemical or mechanical stress during the isolation process can stimulate nuclear leakage, releasing chromosomal DNA. This will cause clumping of the nuclei and thereby result in a decreased yield. The nuclei can also suffer qualitatively from several unwanted biochemical effects during the isolation steps. One of the key steps is centrifugation to separate the nuclei from the cell debris. The density-based ultracentrifugation technique reduces mechanical stress on the nuclei and is more widely used than the standard centrifugation technique.

MedGenome uses a highly reliable and standardized protocol from Miltenyi Biotech that offers recovery of high yield single-nuclei suspension. This workflow has been optimized for a rapid, gentle and effective generation of the single nuclei suspension from the fresh and frozen tissue types.

Figure 1: RNA profiling at single nucleus resolution: a) Tissues are dissociated using gentleMACS optimized protocol and nuclei are isolated, b) Nuclei are stained with Trypan blue and counted on Countess II Automated Cell Counter and Hemocytometer using Olympus CKX53 microscope c) 10X Genomics Single Nuclei Sequencing- Gel Beads-in-Emulsion (GEMs) are created in Chromium Controller based on the Target Nuclei Recovery (500-10,000), d) Sequencing is performed on an Illumina HiSeq/NovaSeq, e) Bioinformatics analysis of the resulting data using 10X Genomics Cell Ranger suite.

Applications

The selection of the single nuclei workflow for the downstream analysis depends on the goal of the project. MedGenome offers several options including ATAC-seq (Assay for Transposase Accessible Chromatin) to analyze chromatin accessibility at the single cell level; 3' gene expression profiling provides and multi-omic profiling (10x genomics).

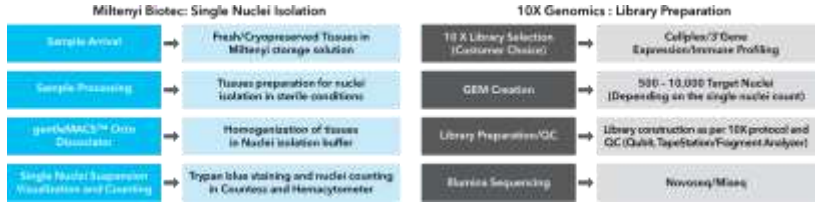


Figure 2: Overview of nuclei isolation and library preparations at MedGenome Inc.

Nuclei Isolation Validation

To validate our protocol, we obtained fresh or cryopreserved mouse brain and liver tissues (120-236mg) in DPBS with 0.04% BSA. Using our optimized protocol, we were able to isolate >3000 nuclei per microliter. The nuclei concentration was assessed using trypan blue staining and visualized under Countess II Automated Cell Counter and Hemacytometer (Figure 3).

S. No.	Species	Tissue Condition	Tissue Type	Tissue Amount (mg)	Isolated Nuclei/µl
1	Mouse	Fresh	Liver	222	5540
2	Mouse	Cryopreserved*	Liver	120	7300
3	Mouse	Fresh	Brain	236	2960
4	Mouse	Cryopreserved*	Brain	201	4280

Figure 3: The Countess II Automated Cell Counter images of the nuclei concentration of the 4 samples

Data Generation

Cell Ranger Single-Cell Software Suite (v5.0.0; 10x Genomics) was modified for analysis of pre-mRNA transcripts. Following sample demultiplexing, barcode processing and quantification of genes, feature-barcode matrices were obtained from cell ranger and subsequently analyzed using Seurat package (v4.0). The cells were filtered for outliers (feature counts) and for high mitochondrial content. Next, cell annotation was performed using SingleR. Cell clustering was performed using a graph-based method using principal components analysis (PCA) and the resulting clusters were visualized using Uniform Manifold Approximation and Projection (UMAP) dimensional reduction representation.

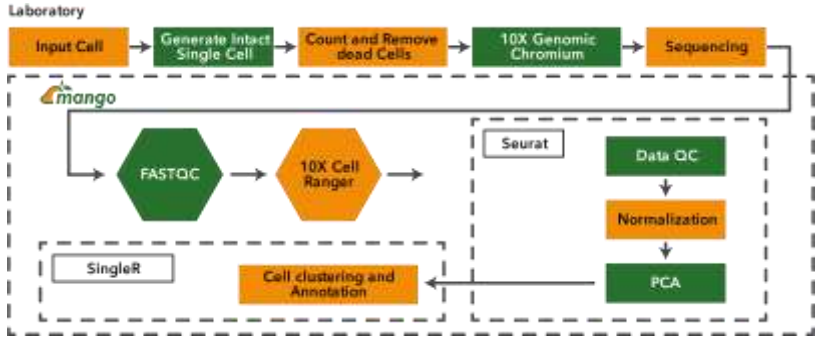
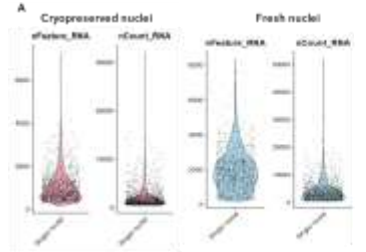


Figure 4: Data analysis workflow

Data Processing



	Cryopreserved nuclei	Fresh nuclei
# Nuclei captured	1,774	1,683
Total # Reads	40,107,508	33,851,287
Percentage of Mapped reads to genome	90.8	94.2
Percentage of Mapped reads to transcriptome	59.0	77.1
Mean # Reads per nuclei	22,609	20,114
Median # of genes	840	1,649

Figure 5: A. Violin plots showing similar number of genes detected per cell (nFeature_RNA) and number of UMIs per cell (nCount_RNA) in nuclei extracted from either fresh or cryopreserved tissues. **B.** Summary statistics of single nuclei sequencing for both sample types.

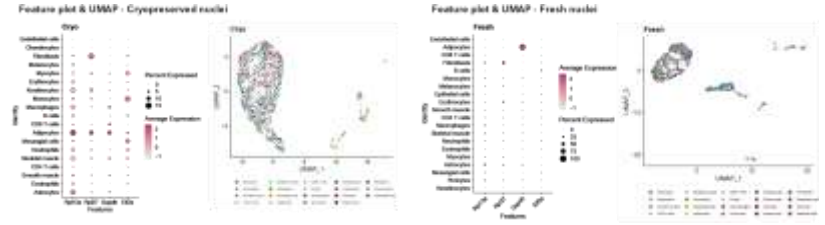


Figure 6: Comparable clustering is observed in fresh and cryopreserved tissues: Putative cell types of each cluster were annotated using top differentially expressed genes within each cluster. Cell types identified in both samples were largely concordant.

snRNA Sequencing Services Highlights

- Flexible starting material accepted including fresh and frozen tissues >150 mg weight.
- Optimized workflows for various tissue types that maximize project flexibility, data accuracy and speed.
- Support with experimental design, end to end solution from nuclei isolation to bioinformatics analysis
- Highest throughput sequencing platforms including Illumina NovaSeq
- Experienced bioinformatics team with standard/custom data analysis offerings

Deliverables

- ✓ FastQ files
- ✓ Mapping metrics
- ✓ Gene Count Files
- ✓ t-SNE plots
- ✓ Hierarchical clustering analysis
- ✓ Differential gene expression analysis files
- ✓ HTML web data analysis summary