Single Cell Solutions at MedGenome

Overview

Single cell genomics is a powerful approach to uncover cellular heterogeneity in normal and disease tissues, and understanding molecular mechanisms of development and disease. However, preparing samples for processing and isolating or capturing a sufficient number of single cells to be able to answer the research question of interest is challenging. The wide ranges of tissue architecture, and cellular sizes and shapes calls for the availability of platforms that can efficiently recover sufficient numbers of single cells for the assays of interest. In addition to the diversity in cell sizes and shapes, there is a wide range of starting numbers of cells and viability that is available for processing. In order to ensure successful generation of single cell libraries from a wide range of starting material, at MedGenome we have integrated several of the well-validated single cell isolation and library preparation platforms.

Workflows for sample processing and selection of single cell genomics applications at MedGenome

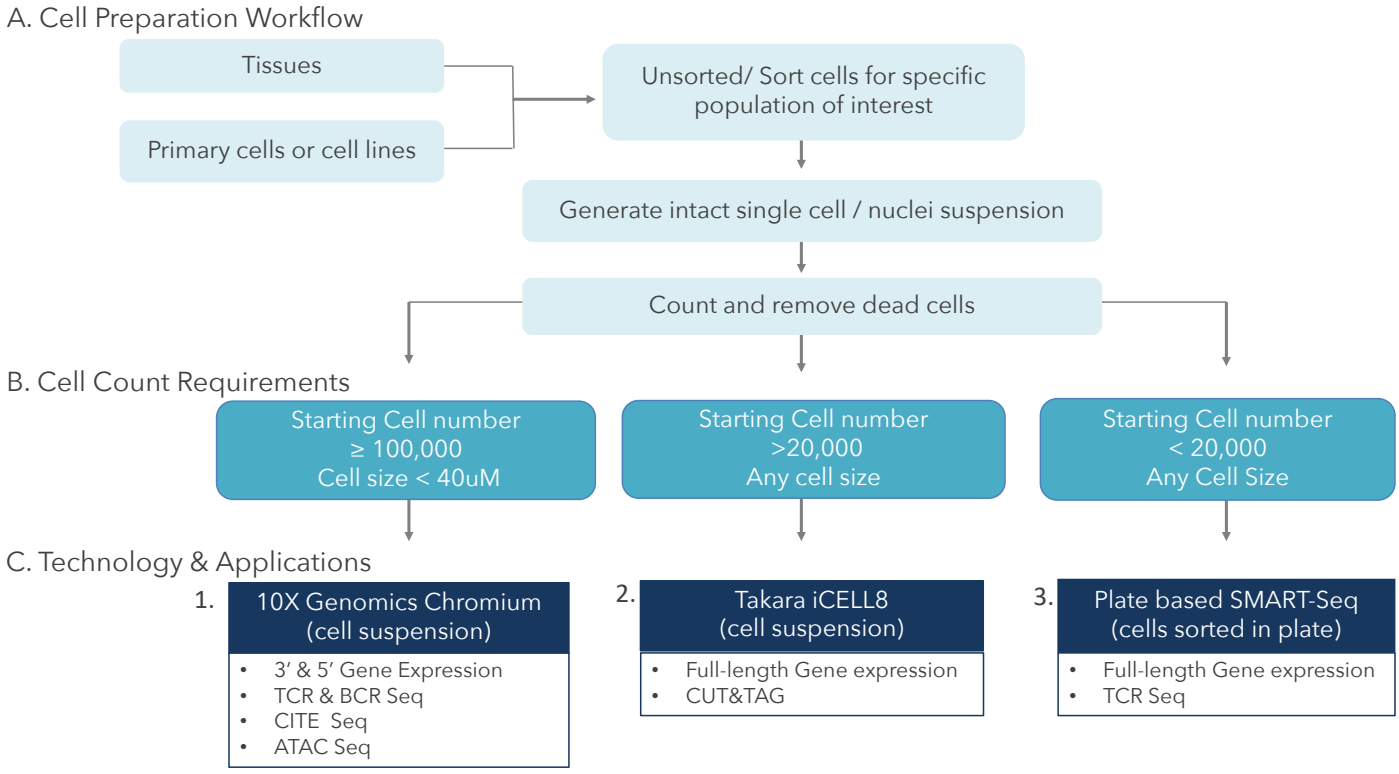
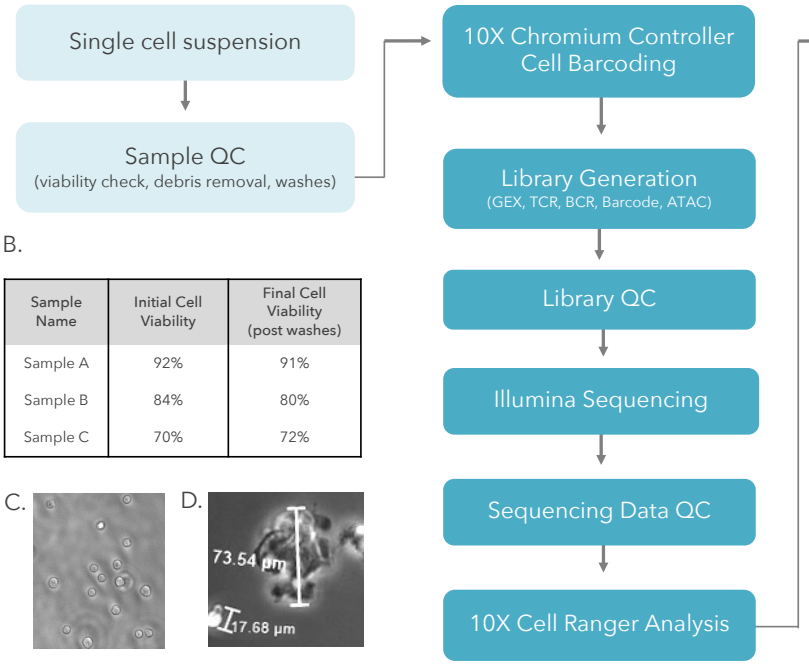


Figure 1 A) Starting with intact single cell / nuclei suspensions obtained from dissociated tissues, primary cells, cell lines or sorted cells, cell viability checks (trypan blue staining and microscopic examination) and dead cell removal steps are performed via washes. B) The rationale for selecting the platform for single cell isolation/encapsulation depends on the starting number of viable cells available, the recovery rate needed and the cell size and shape of the cell. C) At MedGenome, we have integrated and validated three of the well validated approaches. C.1) If there are greater than 100,000 cells available with good viability, and cells are less than 40uM, the 10X Genomics Chromium is recommended. If cells are larger than 40uM , the following two options are available for single cell genomics. C.2) If the application calls for sequencing over a thousand cells, and imaging the cells might be of value, the Takara ICELL8 platform is an ideal platform to select and image single cells. C.3) Lastly if there are a few hundred to thousands of cells available for sequencing, the plate based SMART-Seq technology is an ideal approach to capture the full-length transcriptome diversity.

10X workflow for single cell analysis

A.



B.

Sample Name	Initial Cell Viability	Final Cell Viability (post washes)
Sample A	92%	91%
Sample B	84%	80%
Sample C	70%	72%

C.

D.

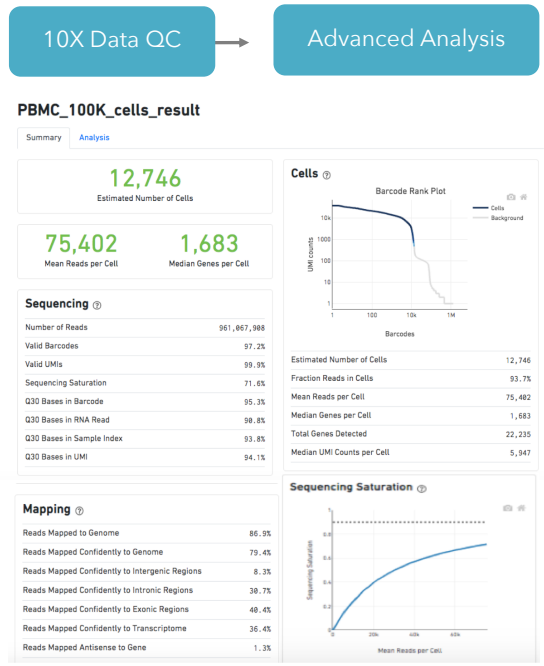


Figure 2 A) 10X Genomics single cell workflow overview. Extensive sample QC is performed prior to proceeding with the library preparation and sequencing. Viability is determined using Trypan blue staining. Before proceeding with processing cells through the 10X Genomics chromium controller, we remove the dead cells using the EasySep™ dead cell removal kit. B) Shows example table with cell viability measurements after thawing fresh frozen samples, and post wash and clean up. C) shows a bright-field image taken of a T Cells where we see an ideal sample with cell size 6-8 µM. D) shows a bright-field image of dissociated mouse thymus tissue (73.54 µM) which is not ideal for processing with 10X Genomics platform. E) Data QC steps are performed using the 10X Cell Ranger analysis pipeline, and the html report generated shows the sequencing quality, % of reads mapping to the genome, and the number of single cells sequenced. The sequencing saturation curve analysis determines whether additional transcriptome diversity would be identified from additional sequencing.

A.

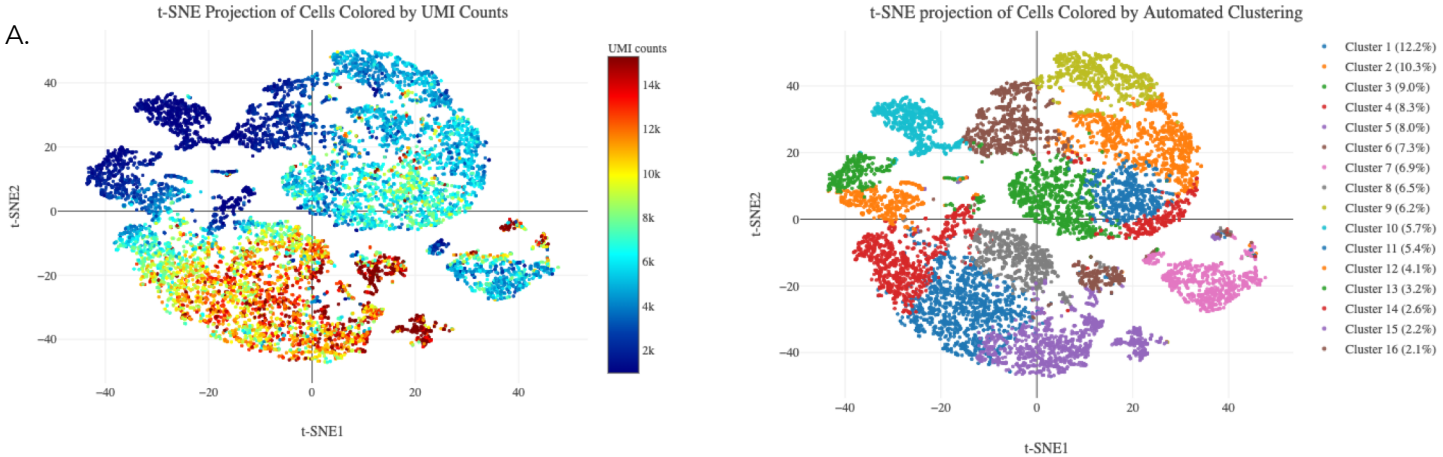


Figure 3 A) 10X Genomics advanced analysis to identify cellular heterogeneity. t-SNE plots shows the clusters identified by cell ranger analysis from a PBMC sample. Additional analysis to identify cell types can be performed by loading the samples onto Loupe browser using marker genes of interest.

Plate-based SMART-Seq workflow for full-length transcriptome profiling

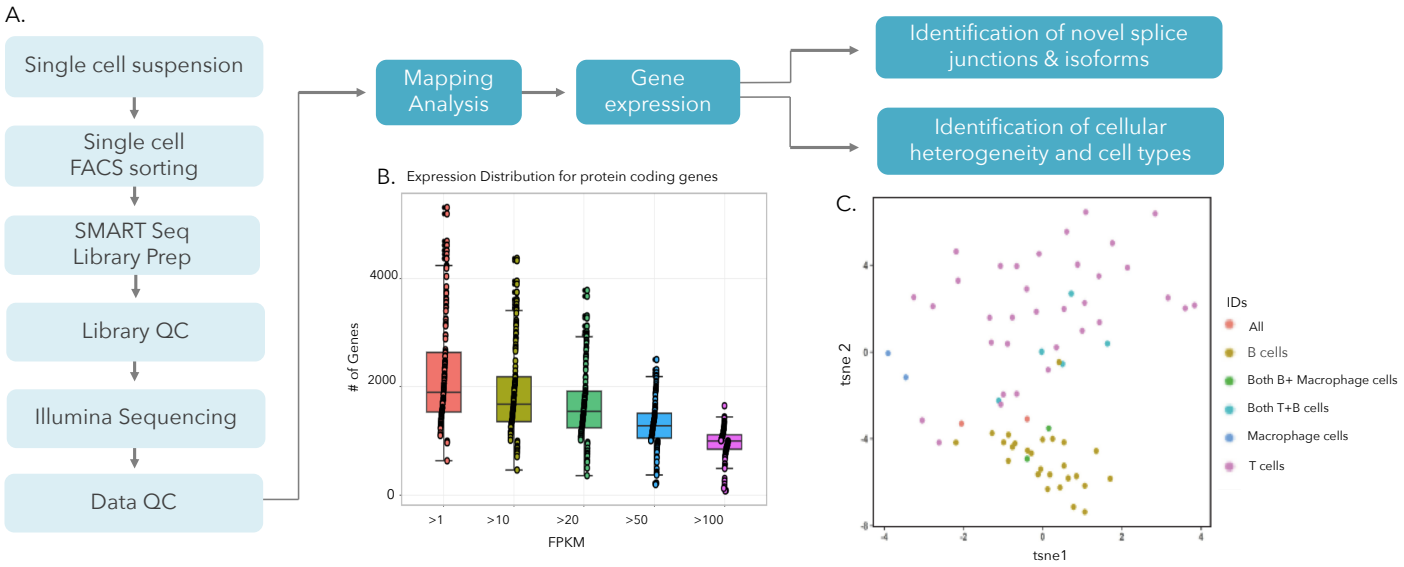


Figure 4 A) Workflow of library preparation for SMART-Seq: Starting from dissociated PBMCs with high viability cells were sorted in Lysis solution and full length SMART-Seq was performed using the Takara SMART-Seq v4 Kit. The libraries were sequenced using NovaSeq 6000 PE100 at a sequencing depth of a 1 million reads per cell. Bioinformatics analysis was performed for mapping and secondary analysis was performed to identify cell types based on gene expression. B) Distribution of the total number of genes per cell with normalized counts shows that at FPKM>1, there are is a median of over 2000 genes identified across the cells with some cells with much greater expression levels per cell. C) Shows tSNE plot with cells that express markers of T cells, B cells and macrophages. By sequencing a 150 cell types from PBMCs with full length chemistry, 3 of the major cell types within PBMCs were identified successfully.

iCell8 Workflow for full length gene expression single cell analysis

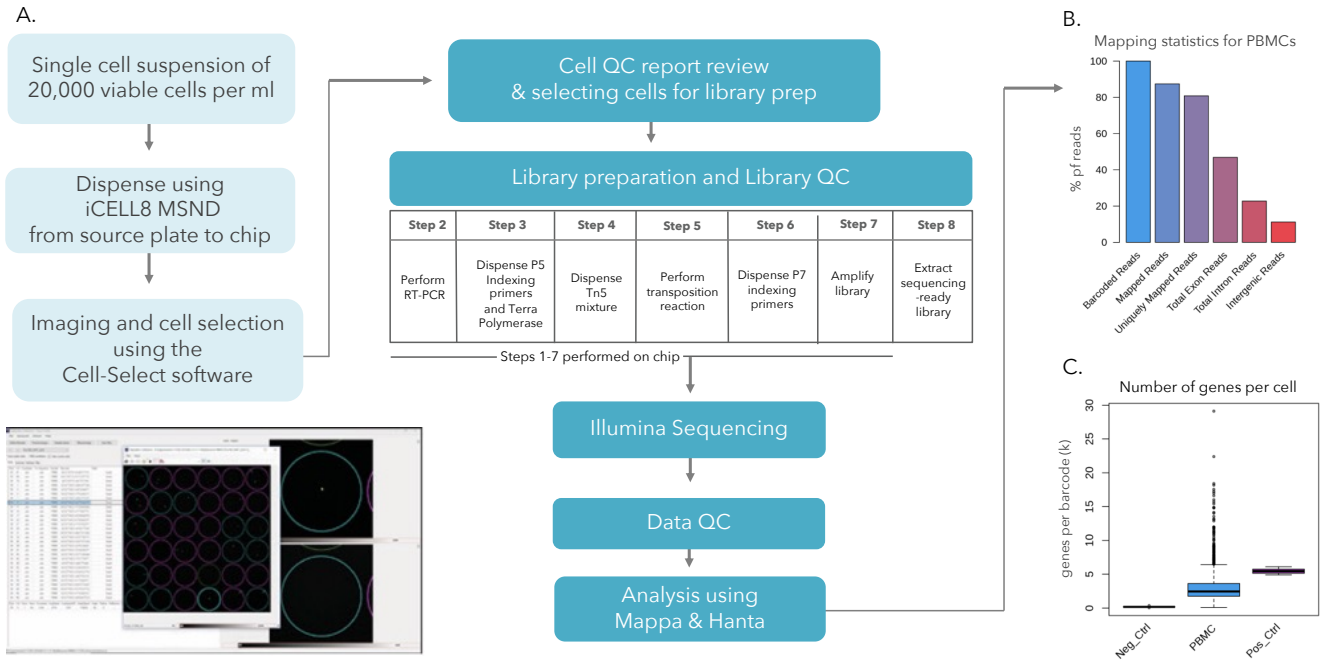


Figure 5 A) iCell8 workflow for sample preparation and library preparation for full-length single cell gene

expression on the Takara ICELL8 platform. Starting with 20,000 PBMCs in a single cell suspension, cells are dispensed into the chip using the MSND (multi-sample nanodispenser). Assessment of live vs dead cells and single cells vs multiplets is completed by fluorescence microscopy based detection. Intact single cells are selected for downstream processing and library preparation. The reagents for library preparation are dispensed and library preparation is completed on the platform with a final step off the platform. The libraries are sequenced at a depth of 300,000 reads per cell using the Illumina NovaSeq and analysis is performed using the Takara Mappa and Hanta analysis software. B) The mapping statistics shows good quality of mapping with 80% or greater reads aligning to the genome, over 40% exonic and low percentage of intergenic reads. C) The median number of genes per cell is 3000, with some cells with over 9000 genes expressed.

Secondary analysis on iCell8 identifies major cell types in PBMCs

A.

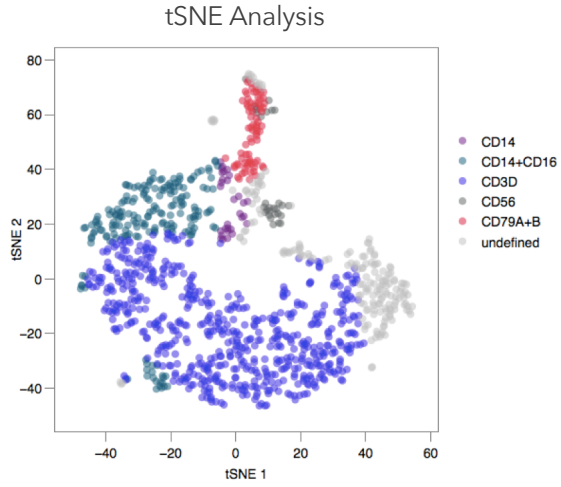


Figure 6 A) t-SNE plot shows identification of the four major cell types (T cells, B cells, monocytes and dendritic cells) using cell type specific marker genes from 1388 live single cells from PBMCs.

Highlights of our Single Cell Services and Bioinformatics Capabilities

- ✓ Support with experimental design and selection of appropriate single cell workflow dependent on cell types and number of cells available
- ✓ Provide end-to-end solution in library preparation, sequencing and bioinformatics analysis
- ✓ Process single cell samples from live, frozen and methanol fixed cells with expertise in sample handling, viability and QC assessment
- ✓ Provide bioinformatics analysis, data interpretation and data curation services

Analysis deliverables

- FastQ files
- Mapping metrics
  Gene body coverage
- Gene Count Files
- tSNE plots
- Hierarchical clustering analysis
- Differential gene expression analysis files
- HTML web summary of the data

Advanced Single Cell Analysis deliverables

- Custom differential analysis and tSNE plots
- Data curation and signature building using singleR and Seurat pipelines (adopted in-house)