Single Cell PLATE Seq Solutions at MedGenome

Overview of PLATE SEQ

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, 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. One of the possible methods to ensure efficient isolation and capture of all single cell from limited material, or subpopulations of cells is to sort into single cell plates and perform a SMART-Seq single cell chemistry to capture the full length transcripts. Furthermore, based on the literature (Lafzi et al, Nature protocols, 2018) , often sampling smaller populations of cells with the full length chemistry downstream of a phenotypic assay can reveal the transcriptional complexity and the cell types in the population. To offer a robust and cost-efficient solution to generating single cell gene expression data from sorted single cells and obtaining maximum transcript diversity, we have validated and incorporated the recent single cell kit from Takara Bio, SMART-Seq single cell, and present validation data on the end to end workflow for the kit.

Experimental Workflow

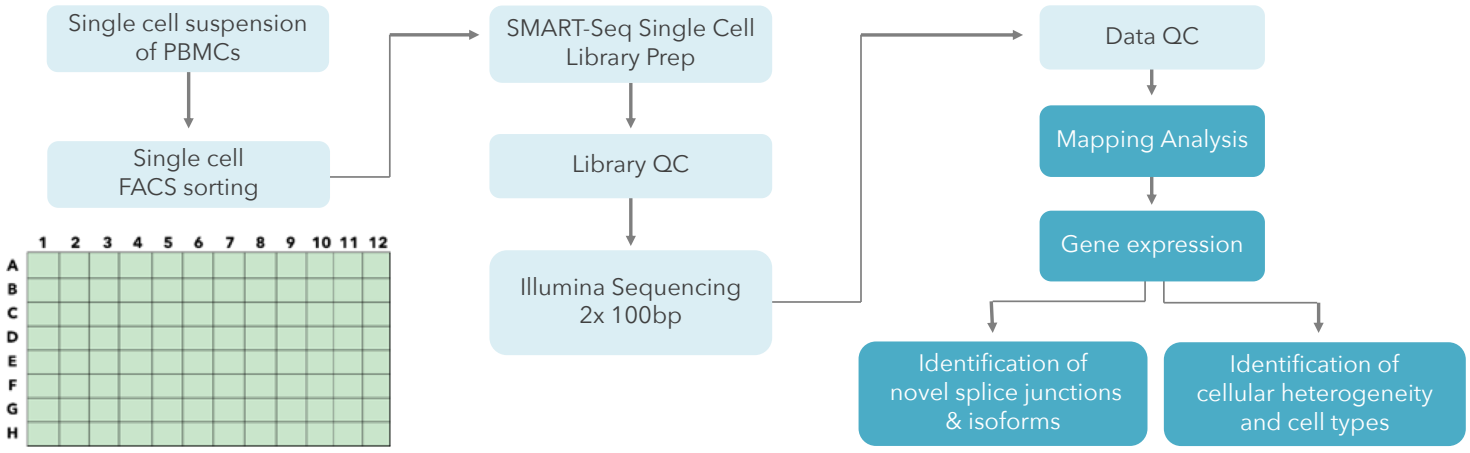

Figure 1: *Schematic representation of the experimental set up to generate single cell plate seq data*
Healthy donor PBMCs were resuspended into a single cell suspension and single cell sorting was performed into 96 well plates. cDNA synthesis was performed using two different single cell kits, SMART-Seq v4 and SMART-Seq single cell Kits (Takara Bio). Cells in wells A1-H1, A2-H2, and A3-H3 were processed using the SMART-Seq v4 (full volume, half and quarter volume respectively), and cells in the remainder of the plate were processed with the SMART-Seq Single Cell Kit (full, half and quarter volume respectively). Sequencing was performed using the Illumina NovaSeq PE 100 Kit. Bioinformatics analysis was performed using the MedGenome pipelines for alignment and gene expression analysis. Downstream of gene expression analysis, we performed secondary analysis to identify the cell types expected to be found in PBMCs.

Results

High quality mapping metrics obtained with SMART-Seq single cell workflow

We analyzed the data obtained from single cell RNA sequencing libraries obtained from the the above described experiment and examined the alignment to the exonic and intronic regions, as well as determined the percentage of ribosomal and mitochondrial reads. We report that we obtained over 80% mapping to the exonic, and low intergenic, ribosomal and mitochondrial. We also compared the data with a gold standard single cell sequencing kit SMART-Seq v4, and found for the cell type tested in our study, PBMCs, the performance of the SMART-Seq single cell kit is equivalent and in some cases depending on the cell type will be more powerful.

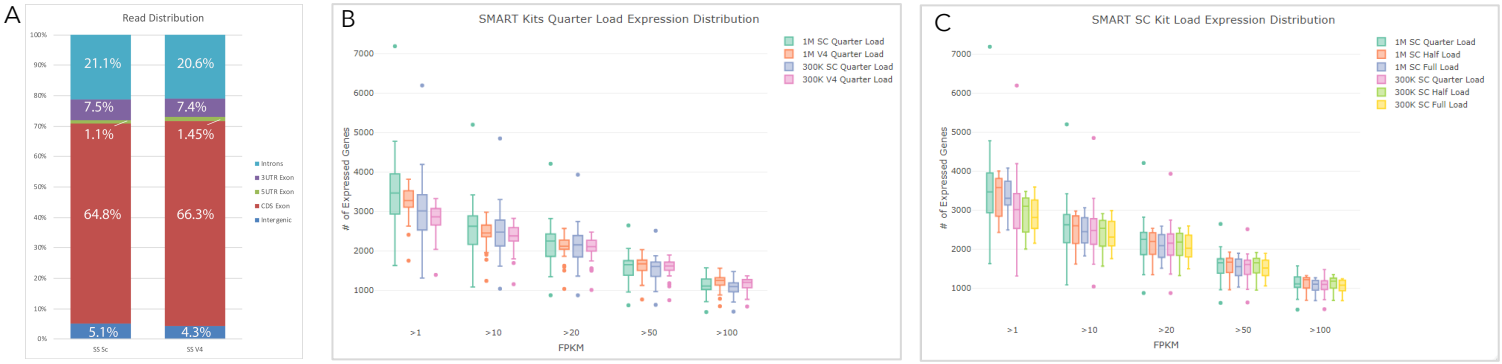

Figure 2: *Alignment and mapping statistics from Takara SMART Seq v4 and Takara SMART Seq single cell kits*
A) Overall statistics shows that the two kits are very similar for the % mapping to exonic, intronic, intergenic and also to the % of ribosomal and mitochondrial contamination. B) Shows a comparison of the distribution of the average number of genes detected per cell using the two approaches and libraries sequenced at different sequencing depths. C) Shows the distribution of the average number of transcripts per cell with cut-offs for the expression levels with the FPKM values listed in the X axis.

Improved performance in the average number of genes per cell for the SMART-Seq single cell Kit
We aligned the raw data to the human genome and compared the alignment statistics between the two kits and found that the % exonic reads are slightly higher in the new single cell workflow, with similar overall low mapping to intergenic, indicating that the level of genomic contamination is low. Furthermore, we found that there was low mapping to ribosomal and mitochondrial suggesting that the new workflow is able to generate good quality transcriptome data from single cells.

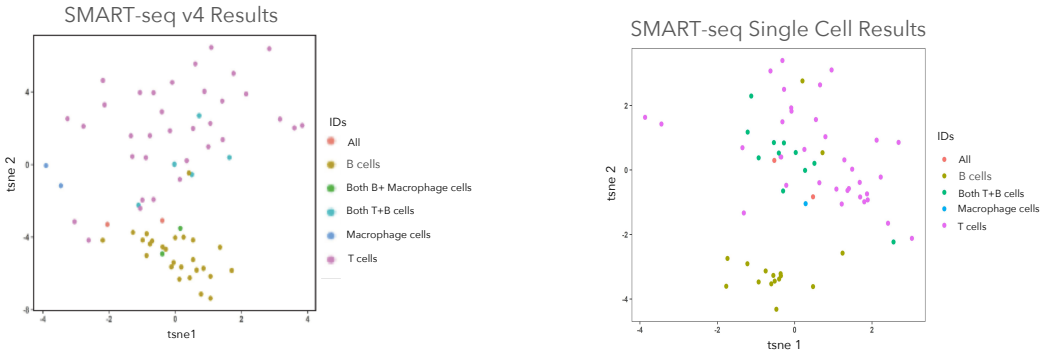

Figure 3) *t-SNE plots of PMBCs show the cell types identified with known markers for expected cell types in transcriptome data for both SMART-Seq v4 and SMART-Seq single cell kit workflows have similar identification of cell types.*

We then asked whether we are able to identify the two major cell types from PBMCs, T and B cells, and queried by expression of the bonafide markers for those cell types (namely CD3D for T cells, CD79a&b for B cells and CD14, CD16 for monocytes). We found that both methods are able to identify major cell types in PBMCs from sequencing a small subset of cells. Taken together, we highlight that the SMART-Seq single cell Kit is a recommended kit for full-length single cell transcriptome profiling from sorted cells.

Services for SMART-Seq Projects

End to End Sequencing Services
- Project Design
- Cell Preparation Support
- Library Prep and Library QC
- Illumina Sequencing and Data QC
- Fastq File Delivery

Basic Analysis Service
- Mapping metrics
- Gene body coverage
- Gene Count Files
- tSNE plots
- Hierarchical clustering

Advanced Single Cell Analysis deliverables
- Custom differential analysis and tSNE plots
- Data curation and signature building using singleR and Seurat pipelines