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Single cell RNA Sequencing for tissue samples processed with microfluidic device platform

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Abstract

Protocol for single cell RNA sequencing of tissue samples processed with a microfluidic device platform.

Troubleshooting

1 Euthanize mice (12 week, male, C57BL/6 for kidney; 10 week, female, MMTV-PyMT for mammary tumor, both from Jackson Laboratory, Bar Harbor, ME) by CO2 inhalation.

2 Dissect kidneys or mammary tumor and mince into ~1 mm3 pieces.

3 Process samples using microfluidic device platform.

4 After device processing, centrifuge recovered cells (400xg, 5 min) and treat with 100 Units of DNase I for 5 min at 37°C, and wash by centrifugation into PBS+1% BSA (PBS+).

5 Incubate samples with RBC lysis buffer for 5 min on ice, centrifuge, and resuspend in PBS+.

6 Stain cells with SytoxBlue (Life Technologies, Carlsbad, CA, USA) prior to FACS (FACSAria Fusion, BD Biosciences, Franklin Lakes, NJ) to remove dead cells and ambient RNA.

7 Centrifuge sorted live single cells (SytoxBlue-neg) and resuspend at a concentration of 1000 cells/µL in PBS with 0.04% BSA. ‘

8 Use the 10x Chromium system (10x Genomics, Pleasanton, CA) for droplet-enabled scRNA-seq. Load oil, cells, reagents, and beads onto an eight-channel microfluidic chip. Load lanes with ~17,000 cells from each sample, as determined using an automated cell counter (Countess II, Invitrogen, Carlsbad, CA).

9 Perform library generation for 10x Genomics Single Cell Expression v3 chemistry according to manufacturer’s instructions.

10 Sequence the samples at the desired depth using an Illumina NovaSeq 6000 platform (Illumina, San Diego, CA).

11 Align sequencing fastq files using 10x Genomics Cell Ranger software (version 3.1.0) to an indexed mm10 reference genome.

12 Use Cell Ranger Aggr to normalize the mapped reads for cells across the libraries for each data set.

13 Discard genes that were not detected in at least 3 cells from further analysis. Discard cells with low (3000 for kidney; >4000 for mammary tumor) unique genes expressed, as

these potentially represent low quality or doublet cells, respectively. Discard cells with high mitochondrial gene percentages (>50% for kidney and >25% for mammary tumor), as these can also represent low quality or dying cells.

14 Use the Seurat pipeline for cluster identification. Perform principle component analysis (PCA) using genes that are highly variable. Perform density clustering to identify groups and use Uniform Manifold Approximation and Projection (UMAP) plots to visualize the groupings.