Washington University - University of California San Diego (WU-UCSD)

(Sanjay Jain and Kun Zhang Labs)

Single-Nucleus Drop-seq (snDrop-seq)

Abstract: snDrop-Seq is a droplet-based method for effective nuclear membrane lysis, capture and sequencing of the RNA molecules in single nuclei. RNA molecules are transcribed and processed within the nucleus before exporting to ER for translation into proteins. As such, nuclear RNA is a mixture of nascent transcripts, partially or fully processed mRNA, and various non-coding RNA molecules. The total RNA content within the nucleus is roughly 10% of the RNA content in a whole cell, but has been found to accurately represent whole cell expression values in adult human tissues [1,2].

snDrop-seq

As per Drop-seq Protocol v.3.1 (Dec 2015) [3,4] with the following modifications [2]:

1. Chemical Reagents:
   a) Nuclei resuspension buffer:

Reagent	Stock	Volume
PBS	1X	699 µl
Ficoll PM-400	20%	300 µl
BSA	10%	1 µl

   b) snDrop-seq lysis buffer:

Reagent	Stock	Volume
H_2O		800 µl
Sarkosyl	20%	10 µl
EDTA	0.5 M	40 µl
Tris pH 7.5	2 M	100 µl
DTT	1 M	50 µl

Note: Add DTT just prior to starting each Drop-Seq Experiment

   c) On page 8 of Drop-seq Protocol v.3.1 (Dec 2015), "Prepare the cells": nuclei instead of cells were prepared with nuclear extraction buffer (NEB). Before droplet generation, connecting tubing and syringes were coated with 1% BSA to prevent non-specific binding of nuclei to the surface, and then rinsed with PBS
   d) On page 9 of Drop-seq Protocol v.3.1 (Dec 2015), “Starting your run”: droplets were collected in 15 mL falcon tubes, were overlaid with 500 µl of mineral oil to prevent evaporation, and then transferred to a 72°C water bath. After 5 minutes of incubation, the tubes were moved from the water bath to ice and the droplets were broken as described on page 11 of Drop-seq Protocol v.3.1 (Dec 2015).
   e) On page 16 of Drop-seq Protocol v.3.1 (Dec 2015), “PCR program”, the cycle number of the second round PCR was increased to 13 for a total of 17 cycles. Total cDNA should be 1-100ng.
   QC cutoff: >1ng.
   f) On page 18 of Drop-seq Protocol v.3.1 (Dec 2015), “Sequencing your sample”, for HiSeq Rapid Run, 8-12 snDrop-seq libraries are combined to make a 10 µl library pool at 3 nM for denaturation. After final dilution, a combined library at 12 pM was loaded to the sequencer

snDrop-seq data processing

1. Mapping, demultiplexing and QC processing was performed as described [5] with modifications [2]. This pipeline is available as a separate software package (https://github.com/chensong611/Dropseq_pipeline). Briefly:
   a) Paired-end reads were removed if read 1 had more than four non-T bases in the last ten bases (to remove all non-poly(T)-captured contaminated reads)
   b) Paired-end reads were removed if read 1 had one or more bases with a poor quality score (<10)
   c) The right mate of each read pair was trimmed to remove any portion of the SMART adaptor sequence or any large stretches of poly(A) tails (6 consecutive bp or larger)
   d) The trimmed reads were aligned to the human genome (GENCODE GRCH38) with STAR v2.5 with default parameter settings
   e) Reads that mapped to intronic or exonic regions as per the GENCODE gene annotation were included in gene counts
   f) Barcode synthesis errors were corrected by inserting N at the last base of the cell barcode for reads in which the first 11 bases of the cell barcode were identical and the last T base of the UMI was the same
   g) UMI counts for each gene of each nucleus were assigned by collapsing UMI reads that had only 1 edit distance to create a digital expression matrix (genes as rows, cells or nuclei as columns)

   QC retention metrics to be applied:
   - ***Nuclei that detect >400 non-mitochondrial transcripts***
   - ***Nuclei that detect >400 and <5000 genes***
   - ***Libraries having > 50 Post-QC nuclei***

   QC preliminary verification:
   - ***From kidney cortex we expect cell types from at least 4 major segments represented out of – glomeruli, proximal tubules, thin loop, distal tubules, collecting system. Also correlate with tissue morphology/composition/quality metrics captured by light microscopy evaluation.***

   QC Reproducibility Assessment:
   - ***Reproducibly will be assessed in part through: 1) the pass/fail rates for snDrop-seq experiments (using the >50 nuclei/library threshold described above); 2) batch effects in gene expression data; 3) expression estimates between technical and/or biological replicates.***

References

1. Lake et al. (2016). *Science*, doi:10.1126/science.aaf1204.
2. Lake et al. (2018). *Nature Biotechnology*, doi:10.1038/nbt.4038.
3. Macosko et al. (2015). *Cell*, doi:10.1016/j.cell.2015.05.002
4. http://mccarrolllab.com/dropseq/Online-Dropseq-Protocol-v.-3.1-Dec-2015.pdf
5. http://mccarrolllab.com/wp-content/uploads/2016/03/Drop-seqAlignmentCookbookv1.2Jan2016.pdf