Single-cell mRNA Seq with Integrated Barcoding
Off chip protocol for Ion Torrent Sequencing:

Required primers:

Upper Transposon Oligonucleotide:
5'-AGA TGT GTA TAA GAG ACA G-3'

Lower Transposon Oligonucleotide:
5'-PhosCTG TCT CTT ATA CAC ATC T-3'

Reverse Library Primer:
5'-CCA CTA CGC CTC CGC TTT CCT C-3'

Forward Library Primer:
5'-CCA TCT CAT CCC TGC GTG TCT C-3'

Extended Reverse Library Primer:
5'-CCA CTA CGC CTC CGC TTT CCT CTC TAT GGG CAG TCG GTG ATA GAT GTG TAT AAG AGA CAG-3'

Required reagents:

Ez-Tn5 Transposase (Lucigen PN TNP92110)
Taps buffer (50 mM TAPS-NaOH, 25 mM $MgCl_2$, pH 8.5 @ RT)
Glycerol
Dimethylformamide (Sigma PN D4551)
Dynabeads MyOne Streptavidin C1 (Thermo Fisher PN 65001)
Dynabead wash buffer (10 mM Tris-HCl pH 7.5, 1 mM EDTA, 2 M NaCl)
SPRIselect beads (Beckman Coulter PN B23317)
85% Ethanol

Protocol

Pool samples for library prep:

1. Combine all material from the C1 harvest (~3.5 µl/cell) in a single 1.5 ml tube. The total volume should be ~336 µl.
2. Add one volume of SPRIselect beads to the pooled harvest material. Wash with 200 µl of 85% Ethanol. Retain beads.
3. Elute bound cDNA with 20 µl water.
4. Quantify cDNA concentration. Final concentration should be at least 1.1 ng/µl.

Transposon Assembly:

1. Combine Upper and Lower transposon oligonucleotides at a final concentration of 50 µM each in TE.
2. In a thermal cycler run the following program to anneal the oligonucleotides:
    - 3 minutes at 95°C
    - 3 minutes at 70°C
    - Cool at 2°C/minute from 70°C to 26°C
3. Dilute 5x with H_2O
4. Add one vol. Glycerol
5. Mix diluted oligonucleotides and Ez-Tn5 transposase (1 U/µl) at a 4:1 (oligo:transposase) ratio and incubate for 30 minutes at room temperature to allow transposon formation.
6. Use immediately or store at -20°C.

Tagmentation:

1. Dilute 10 ng of pooled amplified cDNA in water to a final volume of 9 µl.
2. To the 9 µl diluted cDNA add:
    - 4 µl TAPS buffer (50 mM TAPS-NaOH, 25 mM $MgCl_2$, pH 8.5 @ RT)
    - 2µl dimethylformamide
    - 5µl assembled transposon
3. Mix well and incubate for 7 minutes at 55°C
4. Cool sample to 4 - 10°C or put on ice
5. Add 4 µl of 0.1% SDS and incubate at 65°C for 10 minutes
6. Cool to 4°C

Capture and Library Amplification

1. Wash 24ul of MyOne Streptavidin C1 Dynabeads 3 times with 180 ul 1X Dynabead wash buffer
2. Suspend beads in 24ul 2X Dynabead wash buffer .
3. Add the tagmented cDNA (24ul).
4. Shake or rotate for 15 min at room temperature.
5. Place on magnetic stand, discard supernatant.
6. Wash beads 3x with 180ul 1X Dynabead wash buffer
7. Resuspend washed beads with bound biotinylated fragments in 10.5 µl water
8. To the resuspended beads add:
    - 12.5 µl KAPA HiFi HotStart ReadyMix (2x)
    - 1 µl mix of : 10 µM reverse library primer and 0.5uM extended reverse library primer
    - 1 µl 10 µM forward library Primer
9. Amplify biotinylated fragments with the following conditions:

Temp and Time	Cycles
3 minutes at 72°C	1
30 seconds at 98°C	14
10 seconds at 55°C	
30 seconds at 72°C	
2 minutes at 72°C	1
Hold at 4°C	1

Size Selection (200-350 bp)

1. Add 75 µl TE to 25 µl PCR reaction for a total volume of 100 µl.
2. Add 75 µl SPRIselect beads to the diluted PCR reaction to deplete fragments >350 bp. Wash with 85% EtOH. Discard beads and retain supernatant.
3. Add 15 µl SPRIselect beads to the supernatant from step 2. Wash beads with 85% EtOH. Elute bound cDNA with 10 µl water.
4. Measure library quantity and size using an Agilent Bioanalyzer.