Protocol - Single Cell scRNAseq + scATAC-seq

Luca Lab - December 2022

Name: Date: _____

Project/Experiment: _____

Cell Processing – day 1

Needed for day 1:

- Countess slides
- Trypan blue
- SCAIP media (90% RPMI 1640 +10%CS-FBS+ 0.1% gentamycin)
- 1 round-bottom 96-well plates

Transfer the selected cryovials from liquid nitrogen into dry ice then store in -80C. The day before seeding the plates (Day 1)

Day 1:

1. Thaw one vial of PBMCs in the water bath. Transfer immediately to 6mL of room temperature SCAIP media and mix gently using a wide-bore tip. Rinse the cryovial with an additional 1 ml of media.
2. Count cells on Countess: 10ul cells + 10ul Trypan Blue, Check for viability, and record on the Cell counting sheet page
3. Repeat for 11 additional samples.
4. Centrifuge cells at 400xg for 10 minutes.
5. Resuspend cells to $2x10^6$ cells/mL in a culture medium.
6. Plate 4 wells x 100ul in round-bottom 96-well plates using a wide-bore tip (4wells/individual). Each individual is a separate column.

	1	2	3	4	5	6	7	8	9	10	11	12
A	I_1	I_2	I_3	I_4	I_5	I_6	I_7	I_8	I_9	I_{10}	I_{11}	I_{12}
B	I_1	I_2	I_3	I_4	I_5	I_6	I_7	I_8	I_9	I_{10}	I_{11}	I_{12}
C												
D												
E												
F												
G												
H												

Treat 1, Treat 2

7. Incubate in SCAIP Media overnight at 37C and 5% CO2
8. For each sample, spin the remaining Cells and freeze the pellet for DNA (-80C freezer).

Cell Processing – Day 2

Needed for day 2:

- Treatment media
- Ice-cold PBS
- PBS with 0.04% BSA
- Trypan blue
- 10X Genomics instrument and kits

Protocol:

1. Prepare treatments

Treatments:

LPS: Invivogen Cat# tlrl-3pelps. *lot# 5970-43-03*

- Stock: 5mg/ml *Resuspended stable 1 month at 4°C and 6 months at -20°C.*
- Treatment conc 10ng/ml

Control: Media only

2. Add 2ul of the properly diluted treatments to their respective wells (multi-channel) and mix.
3. Incubate for 6 hrs.

   On ice:
4. Take the plate out of the incubator and pool across rows/individuals (2 pools of 12 individuals):
   - Use multi-channel to gently mix the media using a wide-bore tip and transfer column1(treatment plate) to column 1 of the deep-well plate. Then repeat to transfer columns 2-12 (treatment plate) to column 1 of the deep-well plate. Next, wash columns 1-12 in the treatment plate with 100ul cold PBS then pool to column 2 of the deep-well plate.
5. Pool each row into a 5 ml tube using a wide-bore tip (4 tubes total).
6. Centrifuge @300rcf, 5 min, 4ºC as per the 10X SC Protocol.
7. Remove the supernatant, wash with 5 ml ice-cold PBS+0.04% BSA, and centrifuge again.
8. Resuspend in 1ml ice-cold PBS+ 0.04% BSA.
9. If significant amounts of cell clumps or debris are observed, gently mix cells by pipetting up and down 10 – 15 times and filter cells using a Flowmi Cell Strainer (40 µm).
10. Count cells on the countess, check viability, and record.

Treatment	Cell conc (live)	Viability	Resuspension volume

9. Make sure cell concentration is within the target range of 0.7 M/ml – 1.2 M/ml (aim to capture 25k cells by loading 60K). Adjust if needed. Ideally, viability should be 90% and above acceptable above 80%
10. Proceed with the 10x Genomics® Single Cell Protocols

If you are processing scATAC-seq, take out ~1 million cells in a separate microcentrifuge tube and proceed with the 10x Genomics scATAC-seq protocol.	If you are processing Single Cell Gene Expression, take out the remaining cells e and proceed with the 10x Genomics scRNA-seq protocol.

NOTE:
Ideally, one person will finish with the *Single Cell Gene Expression 10X protocol,* and a 2nd person will handle the *10x Genomics scATAC-seq protocol.*

Single Cell Gene Expression 10X protocols:

https://www.10xgenomics.com/support/single-cell-gene-expression/documentation/steps/sample-prep/single-cell-protocols-cell-preparation-guide

https://www.10xgenomics.com/support/single-cell-gene-expression/documentation/steps/library-prep/chromium-single-cell-3-reagent-kits-user-guide-v-3-1-chemistry-dual-index

10x Genomics scATAC-seq protocol:

Nuclei Isolation for Single Cell ATAC Sequencing:
https://www.10xgenomics.com/support/single-cell-atac/documentation/steps/sample-prep/nuclei-isolation-for-single-cell-atac-sequencing

Samples loading and Lib preparation:
https://www.10xgenomics.com/support/single-cell-atac/documentation/steps/library-prep/chromium-single-cell-atac-reagent-kits-user-guide-v-2-chemistry

Cell counting, viability, and resuspension calculation

Sample #	Sample ID	total cell conc	live cell conc	viability%	Resuspension vol (ml)
1					
2					
3					
4					
5					
6					
7					
8					
9					
10					
11					
12					