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We use this protocol and it's working

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Single cell RNA-seq and ATAC-seq protocol for PBMCs treated with LPS

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ABSTRACT

Single cell RNA-seq and ATAC-seq protocol for PBMCs treated with LPS

ATTACHMENTS

CZI-scRNA-scATAC-protocol.pdf

MATERIALS

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

Needed for day 2:

- Treatment media (See treatments page below)
- Ice-cold PBS
- PBS with 0.04% BSA
- Trypan blue
- 10X Genomics instrument and kits

1 Cell Processing – day 1

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

3 Day 1:

4 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.

5 2. Count cells on Countess: 10ul cells + 10ul Trypan Blue, Check for viability, and record on the Cell counting sheet page

6 3. Repeat for 11 additional samples.

7 4. Centrifuge cells at 400xg for 10 minutes.

8 5. Resuspend cells to 2x106 cells/mL in a culture medium.

9 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.

10 7. Incubate in SCAIP Media overnight at 37C and 5% CO2

11 8. For each sample, spin the remaining Cells and freeze the pellet for DNA (-80C freezer).

12 Cell Processing – Day 2

13 Protocol:

14 1. Prepare treatments (LPS, PHA, Dex, EtOH) – see Treatments page

15 2. Add 2ul of treatments to their respective wells (multi-channel).

16 3. Incubate for 6 hrs.

17 On ice:

18 4. Take the plate out of the incubator and pool across rows/individuals (4 pools of 12 individuals):

19 * 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.

20 5. Pool each row into a 5 ml tube using a wide-bore tip (4 tubes total).

21 6. Centrifuge @300rcf, 5 min, 4oC as per the 10X SC Protocol.

22 7. Remove the supernatant, wash with 5 ml ice-cold PBS+0.04% BSA, and centrifuge again.

23 8. Resuspend in 1ml ice-cold PBS+ 0.04% BSA.

24 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).

25 10. Count cells on the countess, check viability, and record.

26 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%. Proceed with the 10x Genomics® Single Cell Protocols