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Muxab-seq protocol

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Human Cell Atlas Metho...

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Protocol status: In development
We are still developing and optimizing this protocol

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Abstract

This protocol is for staining with AbSeq and multiplexing 16 different samples from distinct individuals into the same 10x run.

Guidelines

Make all necessary preparations for the experiment:

- Notify the IHG core that the 10x Controller will be used.
- Book the Attune NXT for cell counting.

Find the samples the day before (Day 0) and place them in a separate box for easy access the following morning.
Keep frozen PBMC vials on dry ice and thawed cells on watery ice when not in use.
Days do not have to be in order. In general, DNA can be kept at 4°C for < 72 hours and -20°C for < 1 week.

Materials

MATERIALS

Human TruStain FcX™ **BioLegend Catalog #**422301

EasySep™ Buffer 1 L **STEMCELL Technologies Inc. Catalog #**20144

Troubleshooting

Prepare antibody pool

1 Pool all antibodies being used in this experiment.

Thaw Frozen PBMCs

2 Turn water bath on to 37°C, aliquot ~40 mL of EasySep buffer into a tube, and place in the water bath until it comes to temperature.

3 While it warms,
- Get 1 bucket with dry ice and 1 large container with regular ice.
- Get 1 RT Mr. Frosty/Styrofoam box to re-cryopreserve cells after thawing.
- Prepare and label the following: labeled 15-mL Falcon tubes and one U-bottom plate:
- 16 15-mL falcon tube with 5 mL EasySep buffer in each
- 1 U-bottom plate with 16 wells containing 160 uL of flow buffer
- 1 FACS tube with 450 uL of flow buffer

4 Bring all the vials from liquid N2 tank and keep them on dry ice during the preparation time until needed.

5 Thaw two vials at a time, unscrew aseptically and mix gently with P1000 (2x up and down should be enough).

6 Withdraw the appropriate volume containing 2 million cells (as calculated in accompanying worksheet) from each sample's vials and deposit drop-wise into a 15 mL falcon tube with warm EasySep buffer.

7 Close the top of cryovial tightly and place cryovials with the rest of cells into RT Mr. Frosty.

8 Once all vials have been used or closed, move the Mr. Frosty into the -80°C freezer. The next day, move the vials to the LN2 storage.

DNAse Treatment

9 Centrifuge the 16 15-mL tubes at 300 g for 5 min.

10 Pour off supernatant (in one swift motion, being careful not to discard the pellet) and dissociate cell pellet by running the tube on the hood rack. Resuspend the cells in 1 mL of EasySep buffer.

11 Add 1 µl of DNaseI per sample, and mix by swirling. Incubate for 15 min at RT.

12 1.1.1. During incubation, prepare 16 15-mL tubes with 40 um filters on top.

13 1.1.1. Using a P1000, transfer the DNaseI-treated sample onto the 40 um filter and filter through. Wash each tube and filter with an additional 4 mL of EasySep, 1 mL at a time.

Counting on the Attune Flow Cytometer

14 Centrifuge the 16 15-mL tubes at 300 g for 5 min.

15 1.1.1. Pour off supernatant (in one swift motion, being careful not to discard the pellet) and dissociate cell pellet by running the tube on the hood rack. Resuspend the cells in 200 uL of EasySep buffer.

16 Take 10 uL from a random set of 5 samples and add to the FACS tube with flow buffer to use as a control sample for setting voltages on the Attune.

17 Take 40 uL from every sample and place into the corresponding well of the plate that was prepared with flow buffer.

18 Bring the plate and tube to the Attune. Load the tube cells, adjust voltages and draw gates to count the number of cells within the total and live gates.

19 Load the plate and record the samples in the plate, using a 100 uL recording volume, 100 uL per minute flow rate. Record the values of live and total cells in the accompanying workbook.

Pooling and Staining

20 Withdraw the appropriate volume from each sample based on the cell count to achieve a total of 1 million cells. Pool these into one 15-mL tube, and centrifuge at 300 g for 5 min.

21 Pour off supernatant (in one swift motion, being careful not to discard the pellet) and dissociate cell pellet by running the tube on the hood rack. Resuspend the cells in 100 uL

of staining buffer.

22 Add **10µL of FcX blocking reagent**, incubate for **10 minutes on ice.**

23 Add antibody pool to cells.

24 Incubate for **30 minutes on ice.**

Preparing Single Cell Suspension

25 Add 500µL of staining buffer, centrifuge at 300 g for 5 min. Carefully remove supernatant, don't disturb cell pellet.

26 Wash with 1mL **staining buffer** for twice, centrifuge at 300 g for 5 min, remove supernatant.

27 Wash with 1mL **staining buffer** for twice, centrifuge at 300 g for 5 min, remove supernatant.

28 Resuspend pellet in small volume (ie. 100µL of washing buffer), put cell suspension through 40µm cell strainer.
- If use Flowmi strainer, be careful not to push the pipet tip too hard, that breaks the strainer.
- The volume of cell suspension is lost by ~30% with flowmi strianer

29 Take 10µL of filtered cell suspension for counting with Countess in wet lab or ViCell.
- The desired cell density is 2.5 × 10^6 cell/mL (or 2500 cell/µL).
- If cells are too dense, use washing buffer to dilute cells to final density of 2500 cell/µL.
- Use 20µL for each well of 10x library preparation to ensure 10k cells from each sample are loaded.

30 Dilute the cells to 2.5 × 10^6 cells/mL with washing buffer.
- For 2.5 × 10^6 cells/mL, ask the core to load 20 µL for each well.
- Make at least 5µL extra volume for the core to do cell count.

31 Bring cells to IHG core for 10x library preparation.
- Make sure cells are on ice.
- Follow 10X Genomics 3' v2 user guide for running the controller and library preparation.