UPitt TriState SenNet TMC Cell Hashing of single cell suspension for scRNAseq in 5' workflow (10x Genomics)

COMMENTS 0

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WORKS FOR ME 1

TriState SenNet

Cellular Senescence Network (SenNet) Method Development Community

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ABSTRACT

TotalSeq™-C antibodies can be used for Cell Hashing in the 5' workflow, and can be purchased from BioLegend directly. To enable cell hashing, the Demonstrated Protocol for Cell Surface Protein Labeling for Single Cell RNA Sequencing Protocols (10x Genomics) must be modified. The required changes are described below. The recommended number of cells for this modified protocol is 0.2-2 x 10^6 cells per sample.

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PROTOCOL CITATION

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Antibody Mix Supernatant Preparation

1 **Buffers – Preparation**

For samples containing >70% viable cells

- Chilled (4°C): PBS + 1% BSA
- Chilled (4°C): PBS + 0.04% BSA

For samples containing <70% viable cells

- Chilled (4°C): PBS + 10% FBS

2 **Prepare Antibody Mix Supernatant**

• Add appropriate/manufacturer's recommended amount of antibody-oligonucleotide conjugates to a 1.5-ml microcentrifuge tube.

• If using a custom lyophilized antibody: Resuspend the antibody-oligonucleotide conjugates in an appropriate volume of labeling buffer.

• Centrifuge the mix at 14,000 rcf for 10 min at 4°C.

• Transfer the supernatant (containing Antibody Mix) to a new tube and maintain at 4°C

Use TotalSeq-C (Biolegend) for Single Cell 5' v1, v1.1 & v2 protocol with Feature Barcode technology for Cell Surface Protein.

2.1

- TotalSeq™pre-pooled antibody panels, such as the TotalSeq™ Universal cocktails are lyophilized and need reconstitution before proceeding with staining cells.
- Equilibrate the lyophilized panel vial to room temperature for 5 minutes.
- Rehydrate lyophilized panel in PBS + 10% FBS using appropriate reconstitution volume needed for the number cells
- For 0.2-2 x 10^6cells per sample**, add 25-50ul of PBS + 10% FBS to the lyophilized panel. Vortex and incubate for 10min at room temperature. Then, centrifuge the vial at 14000 rcf for 10min at 4°C.
- Transfer the supernatant (containing Antibody Mix) to a low-bind tube and maintain at 4°C.

***Please note: Staining with >500k cells is not covered by BioLegend's product performance guarantee.*

3 **Prepare FACS Antibody Pool**

• Add appropriate/manufacturer's recommended amount of fluorophore antibodies to a 1.5-ml microcentrifuge tube on ice.

• Gently pipette mix and maintain at 4°C. Avoid light exposure.

Cell Surface Protein Labeling Protocol

4 Transfer cells to a new 5-ml tube and add chilled PBS + 0.04 % BSA for a total 1 ml volume.

For samples containing <70% viable cells, use chilled PBS + 10% FBS.

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5 Centrifuge cells at 4°C. Use of swinging-bucket rotor is recommended for higher cell recovery. Centrifugation speed and time depends upon the sample type.

Sample Type	Centrifugation Conditions
Samples containing >85% viable cells, e.g., Pl	300-400 rcf for 5 min
Samples containing <85% viable cells, e.g., tu	150-300 rcf for 5-10 min

Table 2. Sample Type Specific Centrifugation Conditions

6 Remove the supernatant without disturbing the pellet.

7 Resuspend cell pellet in 50 µl chilled **PBS + 10% BSA**.
For samples containing <70% viable cells, resuspend in chilled PBS + 10% FBS.

8 Add 5 µl Human TruStain FcX. Gently pipette mix.

9 Incubate for 10 min at 4°C.

10 Add the prepared Antibody Mix supernatant (TotalSeq™ cell surface antibodies + hashing antibody). Each sample should be stained with a different hashing antibody before pooling the samples.

11 Add chilled PBS + 1% BSA to the cells to bring the **total volume to 100 µl**. Gently pipette mix 10x (pipette set to 90 µl).
For samples containing <70% viable cells, add chilled PBS + 10% FBS.

12 Incubate for 30 min at 4°C. If using FACS antibodies, incubate without light exposure.
Recommended incubation temperature for most sample types is 4°C. However, incubation temperature is sample type dependent and should be chosen accordingly

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13 **Buffers – Preparation**

• Chilled (4°C): PBS + 1% BSA

14 **Wash 1**

Wash by adding 3.5 ml chilled PBS + 1% BSA to the labeled cells. Gently pipette mix.

14.1 Centrifuge at 4°C. Centrifugation speed and time depends upon the sample type. Use Table 2 for guidance.

14.2 Remove the supernatant without touching the bottom of the tube to avoid dislodging the pellet. *Leaving behind excess supernatant may cause non-specific binding, which may result in increased background reads during sequencing.*

15 **Wash 2**

Using a pipette tip, resuspend the pellet or cells in 3.5 ml chilled PBS + 1% BSA and transfer to a new 5-ml tube.

15.1 Centrifuge at 4°C. Centrifugation speed and time depends upon the sample type. Use Table 2 for guidance.

15.2 Remove the supernatant without touching the bottom of the tube to avoid dislodging the pellet.

16 **Wash 3**

Using a pipette tip, resuspend the pellet or cells in 3.5 ml chilled PBS + 1% BSA.

16.1 Centrifuge at 4°C. Centrifugation speed and time depends upon the sample type. Use Table 2 for guidance.

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16.2 Remove the supernatant without touching the bottom of the tube to avoid dislodging the pellet

17 ***(OPTIONAL)***

For enrichment of labeled and viable cells by FACS:

• Based on starting concentration and assuming ~50% cell loss, add an appropriate volume chilled PBS + 2% FBS (including a dead cell marker) to obtain a final cell concentration of 5-10 x 106 cells/ ml and proceed to FACS.

• After FACS, determine cell concentration and viability using an Automated Cell Counter or a hemocytometer.

• Proceed immediately to relevant Chromium Single Cell RNA Sequencing protocols with Feature Barcode technology

18 ***If not performing FACS:***

• Based on starting concentration and assuming ~50% cell loss, add an appropriate volume chilled PBS + 1% BSA to obtain a concentration of 700- 1,200 cells/µl.

• Determine cell concentration and viability using an Automated Cell Counter or a hemocytometer.

• Proceed immediately to relevant Chromium Single Cell RNA Sequencing protocols with Feature Barcode technology.

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