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JMN-MSMP Mouse Muscle Fibrosis scRNA-seq

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Lexi Rindone[1]

[1]Johns Hopkins University

Lexi Rindone
Johns Hopkins University

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We use this protocol and it's working

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Abstract

This protocol was used to generate a CD45+ cell-enriched scRNA-seq dataset of murine muscle fibrosis induced via a volumetric muscle loss injury and synthetic biomaterial (polycaprolactone) implant. This tissue is enriched in p16+ and p21+ cells, enabling the profiling of senotypes involved in muscle fibrosis.

Tissue Harvest and Cell Isolation

1 Euthanize mice according to approved IACUC protocol.

2 Harvest each quadricep from each animal. Take extra care to avoid cutting the femoral artery, as excess bleeding will contaminate the tissue with immune cells from the blood.

3 Place both quads in a petri dish containing chilled RPMI-1640 with L-Glutamine + 25 mM HEPES (Base Media). Keep petri dishes on ice.

4 Dice quads into <3 mm pieces using a razor blade.

5 Add 1:1 v/v of pre-warmed solution of Base Media + 1 mg/mL Liberase TL + 0.4 mg/mL DNAse I (2X Digestion Media) to yield a 1X digestion solution.

6 Incubate quads at 37°C with gentle agitation for 45 min.

7 Add 2:1 v/v of chilled Base Media + 1% BSA to neutralize the Digestion Media.

8 Strain tissues through a 100 µm cell strainer into a 50 mL tube. Use the end of a syringe plunger to force cells through the strainer. Rinse the strainer with 1X DBPS twice, mashing the tissues in between each wash.

9 Centrifuge cells at 400 g for 5 min.

10 Resuspend cells in 5 mL of ACK Lysis Buffer at room temperature for 3 min.

11 Add >20 mL of 1X DBPS and strain cells through a 40 µm strainer.

12 Centrifuge cells at 400 g for 5 min.

Dead Cell Magnetic Bead Removal

13 Resuspend cells in Dead Cell Removal MicroBeads (Miltenyi # 130090101; 10^7 cells/100 µL) for 15 minutes at room temperature.

14 Prepare LS column with 3 mL of 1X Binding Buffer.

15 Add 3 mL of 1X Binding Buffer to cells and add to LS column to enable flow-through of live cells.

16 Collect the live cells from the flow-through solution.

17 Wash column with 4 × 3 mL of 1X Binding Buffer.

CD45+ Cell Enrichment

18 Resuspend cells in 80 µL of Wash Buffer (1X DBPS + 1% w/v BSA + 1 mM EDTA) per 10^7 cells.

19 Add 20 µL of CD45 Microbeads (Miltenyi # 130045801) per 10^7 cells (yielding 100 µL of solution per 10^7 cells) and incubate for 15 minutes at 4°C.

20 Wash cells by adding 2 mL of Wash Buffer per 10^7 cells.

21 Spin down cells at 300 g for 10 minutes.

22 Resuspend cells in 500 µL of Wash Buffer for 10^8 cells.

23 Prime column 3 × 3 mL of Wash Buffer.

24 Add cell suspension to column.

25 Wash column 3 × 3 mL of Wash Buffer.

26 Collect flow-through cell solution. This solution contains the CD45- cell fraction.

27 Remove column from magnet, add 5 mL of Wash Buffer to column, and plunge over a new tube. Collect flow-through cell solution. This is the CD45+ cell fraction.

28 Count cells in the CD45+ and CD45- cell fractions.

29 Combine cell solutions to yield a 1:1 mixture of CD45+ and CD45- cells.

30 Submit samples to sequencing core to perform 10X 3′ Chromium scRNA-seq. (We use the Johns Hopkins Single Cell & Transcriptomics Core.)

Single Cell Sequencing

31 Prepare libraries using 10X 3′ Chromium v3.1 chemistry protocols and reagents.

32 Sequence libraries using NovaSeq platform (Illumina) with S2 100 flow cell at a target depth of 100,000 unique reads/cell.