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scRNA-seq of human femoral bone marrow. V.2

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Kyung J Ahn[1], Shovik Bandyopadhyay[2], Michael Duffy[2], Ling Qin[2], Kai Tan[1]

[1]Children's Hospital of Philadelphia; [2]University of Pennsylvania

Human BioMolecular Atlas Program (HuBMAP) Method Development Community
Tech. support email: **Jeff.spraggins@vanderbilt.edu**

Kyung J Ahn
Children's Hospital of Philadelphia

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We use this protocol and it's working

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Abstract

This protocol describes the methods used for collection and single-cell isolation of human femoral bone marrow cells for single-cell RNA sequencing.

Materials

EasySep RBC Depletion Reagent (STEMCELL Technologies, Cat# 18170)
EasySep Human CD45 Depletion Kit II (STEMCELL Technologies, Cat# 17898)
EasySep Human CD34 Positive Selection Kit II (STEMCELL Technologies, Cat# 17856)
Chromium Next GEM Single Cell 3′ GEM, Library & Gel Bead Kit v3.1, 16 rxns (10x Genomics, Cat# PN-1000121)

Biosample Collection

1 After total hip arthroplasty, femoral head tissue was collected from the Anatomic Pathology Lab after screening for avascular necrosis. Samples were cut in 4-8 mm thick coronal sections on a band saw and placed in a 120 mL collection container with 60 mL of chilled medium (3% fetal bovine serum, 0.1 mM L-acetic acid, 2 mM L-Glutamine and 1% penicillin-streptomycin in MEMalpha).

2 The specimen was transported on ice to the lab.

Cell Digestion

3 The sample is cut into 1-2 mm^3 pieces using sterilized surgical wire cutters and scissors.

4 The minced tissue is weighed and transferred to a 15 mL tube at a volume ratio of 1:2 sample to digestion medium (PBS with 4 mg/mL Dispase II and 2 mg/mL Collagenase I, filtered and pre-warmed) and placed on a 3D-rocker plate in an incubator for 1 hour.

5 The liquid portion of the digested sample is poured through a pre-wetted 100 micron cell strainer.

6 The remaining digested sample is washed 4 times in culture medium (MEMalpha with 15% fetal bovine serum, 1% penicillin-streptomycin, 0.1 mM L-ascorbic acid and 2 mM L-glutamine) and poured through the 100 micron cell strainer.

7 The filtered sample is pelleted at 300 RCF for 10 mins. The supernatant is aspirated, and the sample is resuspended in PBS+2% fetal bovine serum.

8 The resuspended sample is pelleted at 300 RCF for 10 mins.

Single-cell processing for RNA-seq

9 Single-cell isolation is processed per STEMCELL Technologies' protocol for RBC depletion (Cat 18170) using the EasySep system.

10 After RBC depletion, 20 uL is aliquoted for an unenriched faction. The remaining sample is split for rare cell enrichment using STEMCELL Technologies' protocols and EasySep system. For a non-hematopoietic cell enrichment, 2/3rd of the sample is processed with

a CD45 depletion (Cat. 17898). For a hematopoietic stem and progenitor cell enrichment, 1/3rd of the remaining sample is processed with a CD34 selection kit (Cat. 17856).

11 Following processing, each fraction is resuspended at a concentration of 1000 cells/uL in PBS with 0.04% BSA and pooled for scRNA-seq using the Chromium Next GEM Single Cell 3' GEM, Library & Gel Bead Kit v3.1 (10x Genomics, Cat. PN-1000121) and processed per 10x Genomics' protocol.