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Demuxlet Cell Preparation Protocol V.2

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DeMuxlet Cell Preparation Protocol

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Human Cell Atlas Metho...

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GIS

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We use this protocol and it's working

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Abstract

This protocol details Demuxlet cell preparation.

Attachments

DOCX

Demuxlet_Cell_Prepar...

22KB

Guidelines

- If cell viability for any sample is <50%, avoid using that sample in the suspension mix.
- If cell viability is between 50-70%, you may try to enrich for viable cells by centrifuging at lower speed (100 g). It may improve the viability to >75%, the cells can then be mixed with the other samples.

Materials

List of reagents and materials

- RPMI 1640 Medium, no glutamine **Thermo Fisher Catalog #**21870076
- Human Serum **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**H4522
- Fetal Bovine Serum **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**F2442
- L-Glutamine (200 mM) **Gibco - Thermo Fisher Scientific Catalog #**25030081
- Penicillin-Streptomycin (10,000 U/mL) **Thermo Fisher Scientific Catalog #**15140122
- Gibco™ DPBS no calcium no magnesium **Thermo Fisher Scientific Catalog #**14190144
- BSA (Capricorn Scientific; Cat. No.: BSA-1S)
- Axygen™ 1000 µL Universal Pipetter Tips: Wide Bore **Fisher Scientific Catalog #**14-222-703
- Trypan Blue Solution 0.4% **Thermo Fisher Scientific Catalog #**15250061
- MACS® SmartStrainers (30 µm) **Miltenyi Biotec Catalog #**130-110-915
- EVE Cell Counting slides (NanoEnTek, Cat. No. 10027-446)
- QIAamp DNA Mini Kit (Qiagen, Cat no. 51306) QIAamp DNA Mini Kit (250) **Qiagen Catalog #**51306
- Infinium Global Screening Array-24 Kit **Illumina, Inc. Catalog #**20030770
- An appropriate single cell reagent kit and instrument for your application, e.g., 10x Genomics Chromium Controller

Safety warnings

Please refer to the Safety Data Sheets (SDS) for health and environmental hazards.

Preparation of Reagents and Media

1 Prepare appropriate volume of thawing media (RPMI + 5% HS + 1% Pen/Strep + 1% Glutamine) and keep it at 4 °C .

2 Prepare appropriate volume of wash media (RPMI + 10% FBS + 1% Pen/Strep + 1% Glutamine) and and keep it at 4 °C .

3 Prepare appropriate volume of fresh PBS + 0.04% BSA.

Thawing Frozen PMBCs and Preparing the Suspension Mix 1m

4 Warm up thawing media, wash media and PBS + 0.04% BSA in 37 °C water bath.

5 Transfer 9 mL of 37 °C pre-warmed thawing media into each of the 15 mL Falcon tube.

6 Take the cryovial containing PBMCs out of liquid nitrogen storage, place cryovial on dry ice, and transfer immediately to the 37 °C water bath. Thaw for 1 minute- 00:02:00 until no visible ice crystals remain. 2m

7 After thawing for 1 minute- 00:02:00 , open the cryovial in a biosafety cabinet and add 500 µL - 1 mL of pre-warmed thawing media into the cryovial using the 1 mL wide-bore blue tips. 2m

8 After adding the thawing media, use the 1 mL wide-bore blue tips to gently transfer the whole suspension from the cryovial into the 15 mL Falcon tube containing 9 mL of pre-warmed thawing media.

9 Mix the suspension extremely gently by inverting the Falcon tube twice.

10 Centrifuge at 300 x g, 21°C, 00:05:00 .

11 Decant the supernatant.

12 Leave around 200 µL of supernatant behind.

13 Using a serological pipette, gently re-suspend the cell pellet (3-5 times) in 5 mL of pre-warmed wash media.

14 Centrifuge at 300 x g, 21°C, 00:05:00 .

15 Decant the supernatant. Leave around 200 µL of supernatant behind.

16 Using a serological pipette, gently re-suspend the cell pellet (7 times) in 3 mL of pre-warmed PBS + 0.04 % BSA.

Note

Avoid bubbles.

17 Repeat **Step 14-16.**
Centrifuge at 300 x g, 21°C, 00:05:00 . Decant the supernatant. Leave around 200 µL of supernatant behind. Using a serological pipette, gently re-suspend the cell pellet (7 times) in 3 mL of pre-warmed PBS + 0.04 % BSA.

5m

Note

Avoid bubbles.

18 After the second wash, centrifuge at 300 x g for 00:05:00 at 21 °C .
Decant the supernatant until around 200 µL of supernatant is left behind.

5m

19 Gently re-suspend the cells in 800 µL of PBS + 0.04% BSA (pipette ~10 times).

20 Filter the cell suspension through the 30 µm Macs SmartStrainer to remove clumps or debris. After filtering, keep cells On ice .

21 Thoroughly mix 15 µL of cell suspension with 15 µL of trypan blue. Load 10 µL of mixture into each of the two chambers of a cell counting slide.

22 Let the samples sit in the cell counting slide for 00:01:00 before performing cell counting on an automated cell counter. 1m

23 Make aliquots of 1.50 × 10^6 cells/mL for each sample (100 µL aliquot per sample).

24 Keep the remaining cell suspension from individual samples On ice for DNA extraction using a QIAamp DNA Mini Kit (Qiagen, Cat no. 51306) according to the manufacturer's protocol. Perform genotyping using Illumina Global Screening Array-24 v3.0 BeadChip (Cat. No.: 20030770).

25 Mix equal volumes (80 µL) of cell suspension from each sample (up to 16 samples) to make a pooled suspension with a final concentration of 1.50 × 10^6 cells/mL (total volume: 1280 µL). Keep this pooled suspension On ice .

26 Thoroughly mix 15 µL of the pooled suspension with 15 µL of trypan blue. Load 10 µL of the mixture into each of the two chambers of a cell counting slide.

27 Let the samples sit in the cell counting slide for 00:01:00 before performing cell counting on an automated cell counter. 1m

28 Count cells from the pooled suspension using an automated cell counter and verify that the concentration is in the range of 1.10 × 10^6 cells/mL to 1.50 × 10^6 cells/mL.

29 Proceed with single cell capturing: Load 40,000 cells per 10x well using an appropriate 10x reagent kit for your application and run the 10x Genomics Chromium Controller according to the manufacturer's protocol.