Standard operating procedure for single nuclei isolation from frozen bovine ovary and uterus tissue

modified after the Tapestri Nuclei Extraction From Frozen Tissue For Single-Nuclei DNA Sequencing – Mechanical Dissociation; Mission Bio, User Guide, RevE

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AG Genome Annotation

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Purpose

Collecting single nuclei from frozen bovine ovary and uterus tissue. Appropriate preparation of required materials and professional training of lab technicians involved in cell isolation have to be performed in advance.

Materials

- pipettes, pipette tips, wide-bore pipette tips
- LoBind tubes (2, 5 and 15 mL)
- ice and dry ice
- Nuclei Extraction Buffer (Miltenyi, #130-128-024)
- 1X PBS (no Ca/Mg)
- UltraPure BSA (50mg/mL)
- scalpel (sterile)
- petri dish
- dounce set
- cell strainer (70 and 30 µm)
- centrifuge
- AO/PI
- Cellcounter Auto2000 (Nexcelom)
- Hoechst33342
- MoFlo XDP high-speed cell sorter (Beckman Coulter, USA) with an air-cooled Coherent Genesis laser (355 nm, 100 mW).

Preparations

1. Aliquot 2 mL of Nuclei Extraction Buffer (Miltenyi, #130-128-024) into a 5 mL LoBind tube.

2. Prepare 5 mL Nuclei Wash and Resuspension Buffer (4 mL 1XPBS and 1 mL BSA (50mg/mL)).

3. All buffers and reagents are kept on ice or chilled at 4 °C.

4. Prepare a dry ice bucket.

5. For each sample to be processed:

    - Pre-chill on dry ice a pair of sterile scalpels.

    - Pre-chill on dry ice one sterile Petri dish.

    - Pre-chill dounce 2 mL tube and Pestle A and B (Sigma, Part Number: D8938-1SET) in the fridge.

    - Pre-chill a 2 mL LoBind tube.

Nuclei extraction

1. Retrieve cryovial containing the tissue fragment/s from -80ºC freezer and keep on dry ice at all times.

2. Transfer the fragment of tissue into the pre-chilled Petri dish placed on top of the dry ice.

3. Using a chilled disposable sterile scalpel, mince the tissue fragment/s to small pieces.

4. Transfer the tissue to a chilled 2 mL Dounce tube and add 500 µL chilled Nuclei Extraction Buffer to the tube.

5. Dounce with Pestle A until resistance goes away (~10-20 strokes). Dounce with Pestle B for 5-15 strokes.

6. Transfer the homogenate into a 2 mL LoBind tube and add 1 mL of chilled Nuclei Extraction Buffer, mix gently and incubate on ice for 5 min. Gently mix with a wide bore tip and repeat 1-2 times during the incubation.

7. Filter the nuclei suspension through a 70 µm cell strainer and collect the flow through in a 15 mL LoBind tube.

8. Centrifuge the nuclei at 500 x g for 5 min at 4°C, remove supernatant without disturbing the pellet and add 500 µL Nuclei Wash and Resuspension Buffer.

9. Incubate for 5 minutes without resuspending to allow buffer interchange. After incubation, add 1 mL of Nuclei Wash and Resuspension Buffer and resuspend the nuclei.

10. Centrifuge the nuclei at 500 x g for 5 min at 4°C, remove supernatant and gently resuspend nuclei in 1.4 mL Nuclei Wash and Resuspension Buffer.

11. Filter the nuclei suspension through a 30 µm cell strainer and collect the flow through in the 5 mL DNA LoBind tube.

12. Measure the cell concentration and viability using AO/PI staining and the Cellometer Auto2000 (Nexcelom).

Nuclei Sorting

1. Stain DNA via incubation of nuclei with the cell-permeant nuclear counterstain dye Hoechst33342 at a final concentration of 8 µM for 30 min at 4°C.

2. Sort nuclei using a MoFlo XDP high-speed cell sorter (Beckman Coulter, USA) equipped with an air-cooled Coherent Genesis laser (355 nm, 100 mW). Optimal settings to sort the nuclei: 70-µm nozzle at 60 psi in purify mode.

3. Consider events with lower fluorescence intensities as cell debris (Gate A) whereas events with higher fluorescence are nuclei with intact DNA content (Gate B).

4. Collect nuclei in PBS + 0.04% BSA. Measure the cell concentration and viability using AO/PI staining and the Cellometer Auto2000 (Nexcelom).