GENProtocols

Protocol

Detergent-Free Nuclei Isolation

OMICS CANCER RESEARCH DRUG DISCOVERY GENOME EDITING TRANSLATIONAL MEDICINE COMPUTATIONAL BIOSCIENCES

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Abstract

A novel spin column-based approach to isolate intact single nuclei from tissues (fresh or frozen) and cells without using a detergent. The protocol is fast, easy, and reliable, producing high-quality single nuclei with minimized clumping and possible leakage of the nuclear envelope. The protocol is compatible with all single-cell NGS platforms.

Introduction

Single-cell genomics requires high-quality and complete genetic materials from individual cells. However, intact single cells are hard to isolate from tissues, especially when frozen. In addition, Dissociation-induced (either mechanical or enzymatic) population and expression bias are also problematic. It is more feasible to isolate intact nuclei to be used as the starting material for most single-cell genomics applications.

The detergent-free nuclei isolation kit is designed to rapidly isolate intact nuclei from animal cultured cells or tissues (fresh or frozen). Intact nuclei can be isolated from the samples using proprietary spin-column-based technologies in less than 20 min without using tissue homogenizer and any detergents. The traditional method for nuclei isolation involves the use of non-ionic detergent, which has a tendency to cause unwanted nuclear aggregation. It is unclear why some nuclei are aggregated while others are not. It is also unknown whether non-aggregated nuclei are an unbiased representation of the whole nuclei population. Detergents also have the potential to damage the nuclear

envelope resulting in leakage of nuclear matrix materials. For some cell/tissue types, nuclear envelop proteins could also be stripped off by the detergents. Minute™ Detergent-Free Nuclei Isolation Kit provides a whole new way for nuclei isolation in contrast to traditional methods.

Reagents and Equipment

1. 15 ml buffer A (Invent Biotechnologies, Inc. NI-024)
2. 30 ml buffer B (Invent Biotechnologies, Inc. NI-024)
3. 20 protein extraction filter cartridges (Invent Biotechnologies, Inc. NI-024)
4. 20 collection tubes
5. Plastic rod (2) (Invent Biotechnologies, Inc. NI-024)
6. Table-Top Microcentrifuge

Procedure

Cells/tissues are first sensitized by buffer A before passing through the proprietary filter in a zigzag manner when high-speed centrifugal force is applied. The cells are ruptured when passing through the filter leaving intact native nuclei in the flow-through. The nuclei are separated from other small cell debris by low-speed centrifugation using the proprietary buffer B. The native and intact nuclei isolated can be used for a variety of downstream applications that include but not limited to: FACS analysis, single nucleus analysis (such as RNA-seq and ATAC-seq), immunofluorescence staining, cell cycle analysis, and/or apoptosis research.

Nuclei Isolation Protocol for Cultured Cells:

(Pre-chill buffers on ice)

1. Collect 10-50 million cultured cells by low-speed centrifugation (500 X g for 5 min). Wash the cell pellet once with 1 ml cold PBS. Remove the supernatant completely.
2. Resuspend the cell pellet in 500 µl cold buffer A and incubate on ice for 8-10 min. After incubation, vortex the tube vigorously for 20-30 seconds. Transfer the cell suspension to a filter cartridge with a collection tube.
3. Centrifuge in a tabletop microfuge at ~16,000 X g for 20 seconds, resuspend the pellet by pipetting up and down a few times and re-pass the cells through the filter one more time.
4. Discard the filter and resuspend the pellet by vortexing vigorously for 10 seconds, centrifuge at 500 X g for 2 to 4 min (smaller nuclei

require longer centrifugation time). Remove and discard the supernatant.

5. Resuspend the nucleus pellet in 0.5-0.8 ml cold buffer B, centrifuge at 600 X g for 8-10 min (this is to remove membrane debris). The pellet contains isolated nuclei.

Nuclei Isolation Protocol for Mammalian Tissues:

(Pre-chill buffers on ice)

1. Add 20-30 mg fresh or frozen **soft tissue** to the filter cartridge. For frozen tissues, thaw them out completely on ice. **For muscle tissues,** place the tissue on the surface of a clean glass or plastic plate, mince the tissue with a sharp blade into tissue slurry or past. Transfer the tissue to the filter cartridge.

2. Add 200 µl cold buffer A to the filter, grind the tissue for about 1-2 min using the plastic rod provided (the plastic rod is reusable, clean by washing with water). Double the grinding time if muscle tissue is used.

3. Add 300 µl cold buffer A to the same filter, incubate on ice for 5-10 min with the cap open.

Cap the tube and resuspend the tissue homogenate by inverting the tube a few times.

4. Centrifuge in a tabletop microfuge at 16,000 X g for 20 seconds (optional: resuspend the pellet by vortexing and re-pass the pass-through through the same filter one more time).

5. Discard the filter and resuspend the pellet by vortexing vigorously for 10 seconds, centrifuge at 500 X g for 2-3 min. Discard the supernatant.

6. Resuspend the pellet in 0.5-0.8 ml cold buffer B, centrifuge at 600 X g for 8-10 min (this is to remove membrane debris). The pellet contains isolated nuclei.

Further Cleanup of Isolated Nuclei:

For most samples, the isolated nuclei are clean enough for downstream applications. Further cleanup may be considered if contamination of cellular debris is a concern. The following protocol can be used for cleanup:

1. Resuspend nuclei pellet from above procedures in 100-200 µl buffer B.
2. Add 10-20 µl 1% NP-40 to the nuclear suspension (final NP-40 concentration is 0.1%). Mix well and incubate on ice for 3-4 min.
3. Centrifuge at 800 X g for 5 min. The pellet contains cleaner nuclei.

NOTE: This step is application dependent. It may be beneficial if the exposure of detergent to isolated nuclei is not a concern (such as ATAC-seq). It is not recommended for single nucleus RNA-seq for reasons mentioned in the description section above. Please note that due to variations in lipid composition of isolated nuclei from different tissues, cleanup by NP-40 may cause lysis of the nuclei.

Storage of Isolated Nuclei:

Isolated nuclei can be resuspended in a tissue culture medium that contains 5-10% FBS or BSA and stored at 4^{o}C for a few days without significant change in morphology. For long-term storage resuspend the nuclei in 0.5 ml buffer B and store them at -70-80^{o}C. Alternatively, Minute™ Anti-Clumping Nuclei Storage Buffer **(Cat# WA-014)** can be used.

Time Taken

20 min

References

None