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## 🌐 Nuclei Isolation from Human Subchondral Bone for 10x Multiome

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## Abstract

This protocol describes isolation of nuclei from fresh-frozen human knee subchondral bone for use in Omics analyses, including RNA-sequencing of ATAC-sequencing. Tissue dissociation was the most critical step in optimization which was monitored by nuclei yield, integrity and purity. Methods for quality control are also described.

## Troubleshooting

## **Nuclei Isolation from Human Subchondral Bone for 10x Multiome**

### Materials

- 5-ml centrifuge tubes (Eppendorf, catalog number: 0030108310)
  - 50-ml conical centrifuge tubes (Falcon, catalog number: 352098)
  - Cell strainers, 40- $\mu$ m (Fisher, catalog number: 22363547)
  - Cell strainers, 20- $\mu$ m (pluriSelect, catalog number: 43-50020-03)
  - Cell strainers, 10- $\mu$ m (pluriSelect, catalog number: 43-50010-03)
  - Connector Ring (pluriSelect, catalog number: 41-50000-03)
  - Mortar and Pestle
  - Liquid Nitrogen (LN)
  - Ladle for LN handling
  - Ultracentrifuge tubes (Beckman Coulter, catalog number: 344059)
  - RNase inhibitor (Sigma Aldrich, catalog number: 3335402001)
  - Recombinant Albumin solution (New England Biolabs, catalog number: B9200S)
  - PBS (Thermo Fisher Scientific, catalog number: 10010023)
  - DTT, Sigma-Aldrich, Catalog No. 646563
  - Tween 20 (Fisher, catalog number: BP337500)
  - Nonidet P40 Substitute (Sigma Aldrich, catalog number: 74385)
  - Acridine Orange/Propidium Iodide Stain (Logos Biosystems, catalog number: F23001)
- 
- Nuclei buffer (10 $\times$  genomics)
  - Lysis Buffer (see Recipes)
  - Wash Buffer (see Recipes)
  - Diluent Buffer (see Recipes)
- \*Diluent buffer in Distilled Nuclease free water and sterile filtered before use (see Recipes)

Lysis Buffer	Stock	Final	20 ml
Tris-HCL (pH 7.4)	1 M	10 mM	200 ul
NaCl	5 M	10 mM	40 ul
MgCl <sub>2</sub>	1 M	3 mM	60 ul
Tween-20	10 %	0.01%	20 ul
Nonidet P40 Substitute	10 %	0.05%	100 ul
Recombinant Albumin	20 mg/ml	0.3 mg	300 ul
DTT	1 M	1 mM	20 ul
RNase inhibitor	40 U/uL	1 U/uL	500 ul
Nuclease-free water			18760 ul

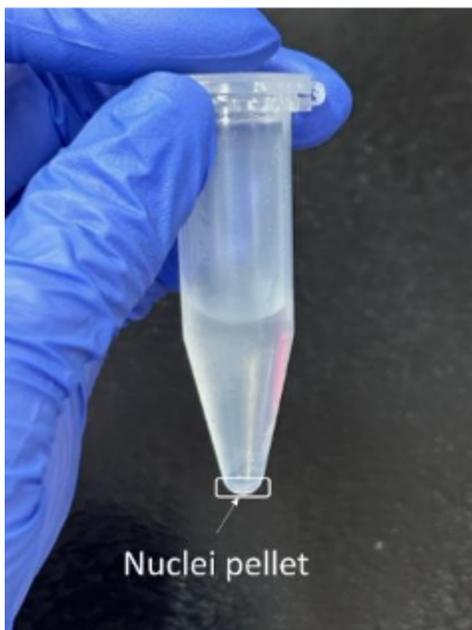
Wash buffer	Stock	Final	20 ml
Tris-HCL (pH 7.4)	1 M	10 mM	200 ul
NaCl	5 M	10 mM	40 ul
MgCl <sub>2</sub>	1 M	3 mM	60 ul
Recombinant Albumin	20 mg/ml	0.3 mg/ml	300 ul
Tween-20	10%	0.10%	200 ul
DTT	1 M	1 mM	20 ul
RNase inhibitor	40 U/ul	1 U/ul	500 ul
Nuclease-free water			18680 ul

Diluted Nuclei Buffer	Stock	Final	1 ml (ul)
Nuclei Buffer (20X)	20X	1X	50 ul
DTT	1 M	1 mM	1 ul
RNase inhibitor	40 U/ul	1 U/ul	25 ul
Nuclease-free Water			924 ul

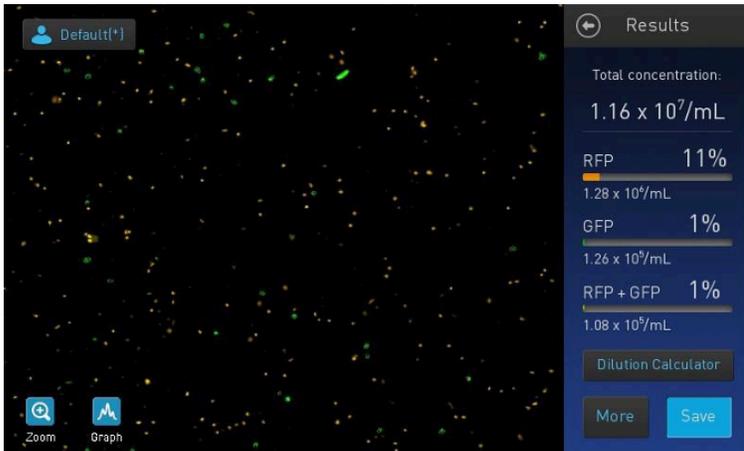
## Nuclei Isolation Protocol Using the Singulator S200

We use fresh snapfrozen tissues stored in Liquid Nitrogen (LN).

- 1.1. Pre-chill mortar and pestle with LN. Place 200mg snapfrozen bone into a mortar, add LN and smash (do not grind) several times with a pestle until it is dissociated. Make sure that the tissue is in tiny fragments while trying not to grind it, as grinding creates more nuclei damage.
- 1.2. Transfer tissue fragments to a 50 ml tube with 2 ml lysis buffer and incubate for 20 min at 4°C.
- 1.3. After this incubation, add 2 ml wash buffer to the tube.
- 1.4. Pass the nuclei suspension sequentially through 40  $\mu\text{m}$ , 20  $\mu\text{m}$ , and 10 $\mu\text{m}$  strainers into a 50-ml conical centrifuge tube on ice. Do not apply negative pressure.
- 1.5. Transfer the flow-through to a 5 ml LoBind tube.
- 1.6. Centrifuge at 500 rcf for 5 min at 4°C.

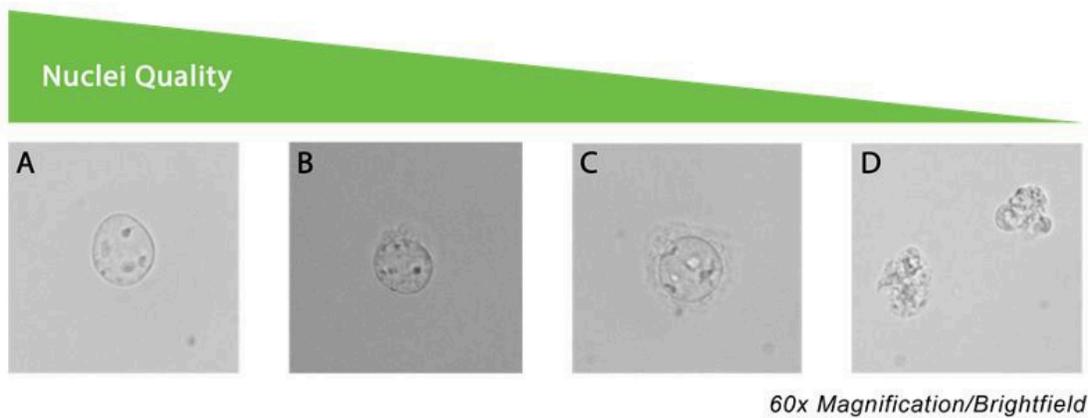


- 1.7. Remove supernatant carefully and resuspend in 400 $\mu\text{L}$  PBS + Recombinant Albumin 0.03 mg/ml + 1U/ $\mu\text{l}$  RNase Inhibitor.
- 1.8. To remove cellular debris, spin down at 4°C, 150 rcf for 10 min to pellet the nuclei and retain the small debris in the supernatant.
- 1.9. Remove supernatant and resuspend in 400 $\mu\text{L}$  PBS + Recombinant Albumin (0.03mg/ml) + 1U/ $\mu\text{l}$  RNase Inhibitor.
- 1.10. Count nuclei using a Countess II FL Automated Cell Counter (AOPI staining).



Example of AOPI counts: Nuclei stained in RFP (orange), live cells and debris stain in GFP (green).

1.11. Nuclei should be visualized under a microscope at 40x or 60x magnification (AOPI staining) to assess nuclear membrane integrity, according to the nuclear integrity quality check guidance by 10x (<https://10xgenomics.com>).



- A: High-quality nuclei have well-resolved edges. Optimal quality for single-cell gene expression libraries.
- B: Mostly intact nuclei with minor evidence of blebbing. Quality single-cell gene expression libraries can still be produced.
- C: Nuclei with strong evidence of blebbing. Proceed at your own risk.
- D: Nuclei are no longer intact. Do not proceed!

1.12. Centrifuge at 500 rcf for 5 min at 4°C and remove the supernatant without disrupting the nuclei pellet.

1.13. Based on the nuclei concentration estimated at step 1.9, resuspend nuclei pellet in chilled Diluted Nuclei Buffer to a target concentration of 4,000 – 5,000 nuclei/ul, for a target nuclei recovery of 20,000 nuclei.

\* Put only 5 µL into the PCR tube

1.14. Proceed immediately to Chromium Next GEM Single Cell Multiome ATAC + Gene Expression User Guide (CG000338) and minimize the time between nuclei preparation and chip loading.