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## Nuclei Isolation from Human Synovium and Infrapatellar Fat Pad for 10x Multiome

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## Abstract

This protocol describes isolation of nuclei from fresh-frozen human knee synovium and fat pad 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.

## Nuclei Isolation from Human Synovium and Infrapatellar Fat Pad for 10x Multiome

### Materials

5-ml centrifuge tubes (Eppendorf, catalog number: 0030108310)

15-ml conical centrifuge tubes (BioPioneer, catalog number: CNT-15)

50-ml conical centrifuge tubes (Falcon, catalog number: 352098)

Cell strainers, 40-µm (Fisher, catalog number: 22363547)

Cell strainers, 20-µm (pluriSelect, catalog number: 43-50020-03)

Cell strainers, 10-µm (pluriSelect, catalog number: 43-50010-03)

Connector Ring (pluriSelect, catalog number: 41-50000-03)

### Forceps

Scalpel (Fisher, catalog number: 72042-21)

Petri dish (Fisher, catalog number: FB0875713)

### Dry ice

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 (10x genomics)

Nuclei Isolation Cartridge, S2 Genomics, Part No. 100-063-732

Lysis Buffer (see Recipes)

Wash Buffer (see Recipes)

Diluent Buffer (see Recipes)

### Singulator instrument and reagents:

Singulator S200 (S2 Genomics)

\*We use a custom *Low Volume* Nuclei Isolation Protocol on Singulator

\*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
Digitonin	5%	0.001%	4 uL
Tween 20	10%	0.01%	20 uL
Nonidet P40 Substitute	10%	0.01%	20 uL
Recombinant albumin		0.3mg/ml	
DTT	1 M	1 mM	20 uL
RNase inhibitor	40 U/uL	1 U/uL	500 uL
Nuclease-free Water			17136 uL

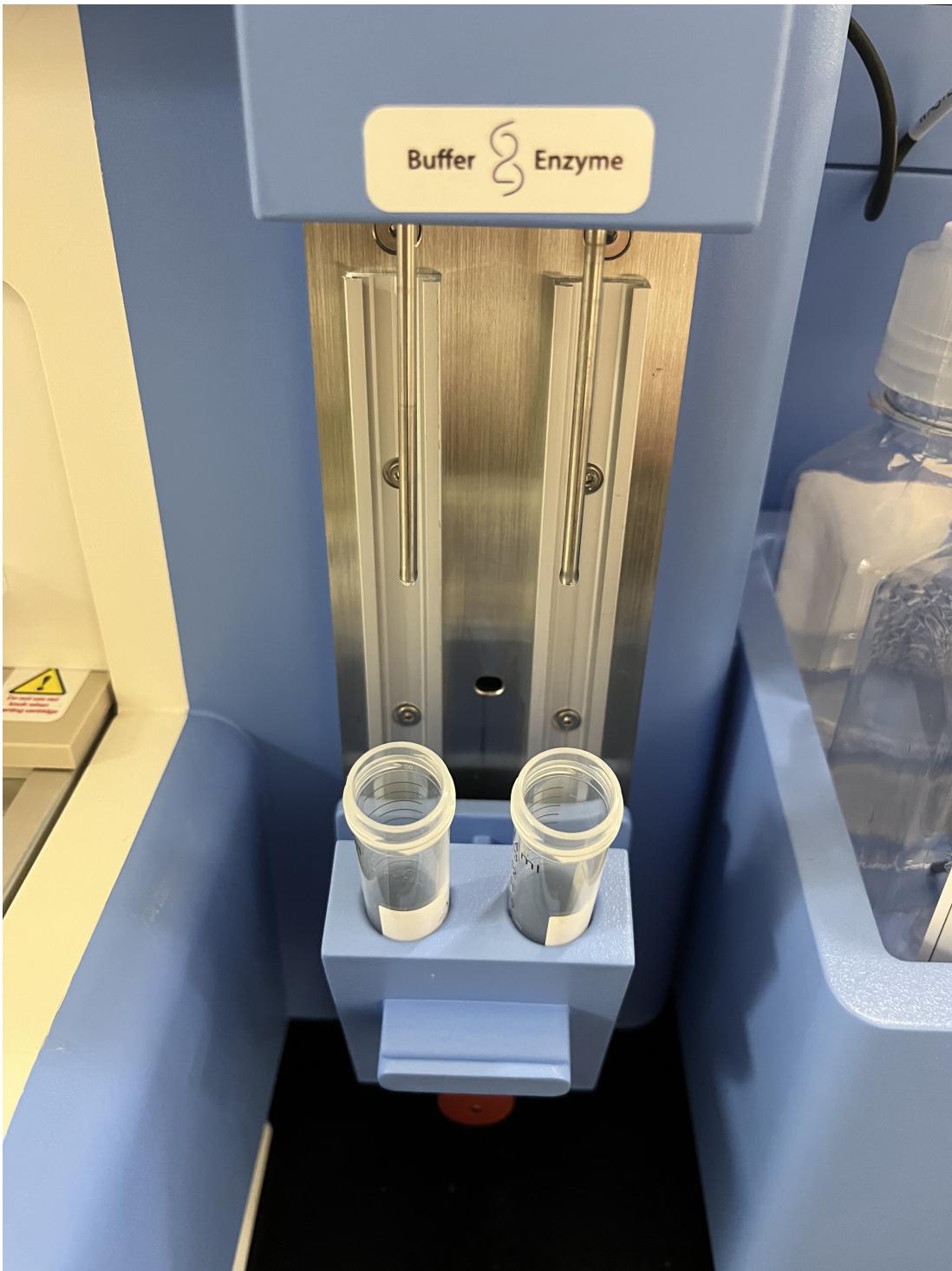
Wash buffer	Stock	Final	20 ml
Tris-HCL (pH 7.4)	1 M	10 mM	200 uL
NaCl	5 M	10 mM	40 uL
MgCl <sub>2</sub>	1 M	3 mM	60 uL
Recombinant albumin		0.2mg/ml	
Tween 20	10%	0.10%	200 uL
DTT	1 M	1 mM	20 uL
RNase inhibitor	40 U/uL	1 U/uL	500 uL
Nuclease-free Water			16980 uL

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

The Singulator S200 Automated Tissue Dissociation System (from S2 Genomics) is programmable and thus allows reproducible, operator-independent nuclei preparation. We use fresh snapfrozen tissues stored in Liquid Nitrogen.

1.0. The buffer recipes are prepared fresh for each nuclei experiment. These custom reagents are pre-chilled and loaded into the Singluator Single Shot holder. The Single Shot holder has two 15 ml conical tube slots for each of the S200 bays of the Singulator. One slot is labeled "Buffer" where we load the Washing Buffer, the other slot says "Enzyme" where we load the Lysis Buffer. Each 15 ml conical tube contains 3 ml of the buffers in their respective tubes.



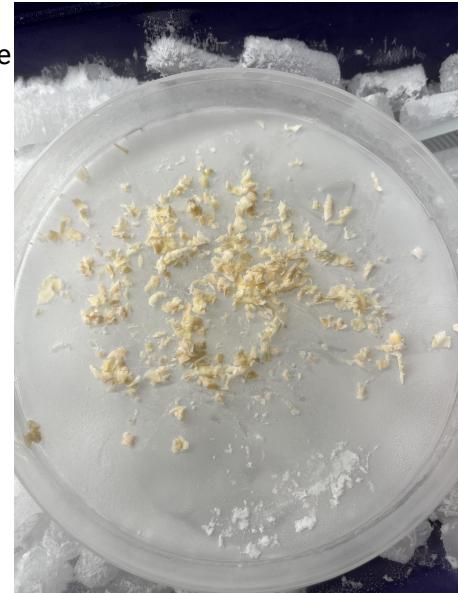
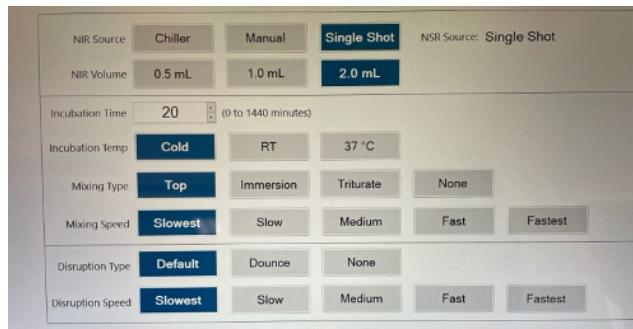
Single Shot holder

1.1. Prior to loading 100-150mg of tissue into to the Singulator cartridge, it needs to be finely shaved and minced into small pieces using a scalpel. The tissue is minced on a petri dish kept on dry ice.

\* Some tissues may become sticky when the temperature rises, so the petri dish to be used should also be cooled. Use LN if needed. Scalpel and forceps should be pre-cooled on dry ice to avoid thawing of the tissue fragments.

1.2. Transfer tissue fragments to a pre-cooled Singulator Nuclei Isolation Cartridge (kept at -20°C), add 75  $\mu$ L of RNase inhibitor (40 U/ $\mu$ L) directly into the disruption chamber of the Nuclei Isolation Cartridge. Using the custom *Low Volume* nuclei isolation program, the final volume in the cartridge is 3 mL, containing 3,000 units of RNase inhibitor total.

1.3. Run the Low Volume custom program on the Singulator:



1.4. Remove cartridge from Singulator, puncture the aluminum seal to collect the nuclei suspension. Pass this suspension through 20  $\mu$ m and 10  $\mu$ m strainers into a 50-mL LoBind conical tube on ice.

If the pestle is difficult to remove, due to negative pressure, let the pressure resolve and then remove the pestle. If some tissue homogenate is left in the tissue loading compartment, you can aspirate with a pipette and pass through a 40  $\mu$ m strainer. Then combine this with the nuclei fraction that had passed through the 40  $\mu$ m filter in the Singulator and pass through 20  $\mu$ m and 10  $\mu$ m strainers to remove tissue and cellular debris.

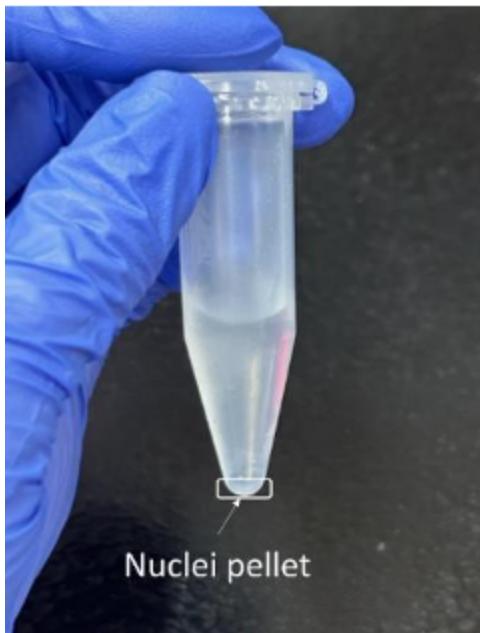
1.5. Transfer the flow-through to a 5 mL LoBind tube.

1.6. Centrifuge at 150 rcf for 10 min at 4°C.

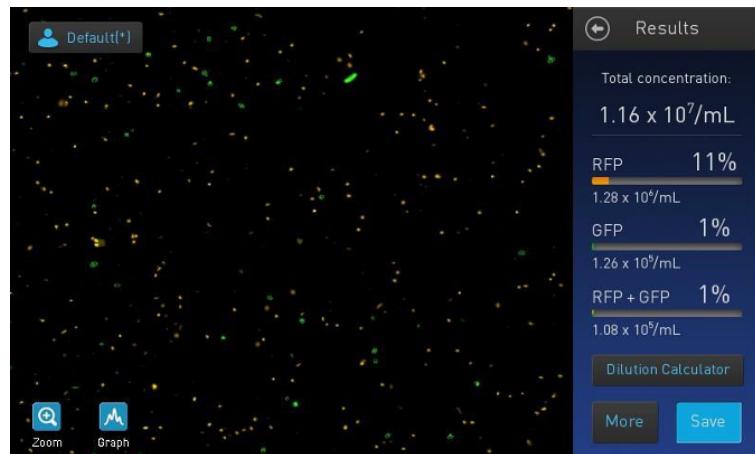
1.7. Remove supernatant carefully and resuspend in 600 $\mu$ L PBS + Recombinant Albumin 0.02 mg/ml + 1U/ $\mu$ L RNase Inhibitor.

1.8. To remove cellular debris, centrifuge at 4°C, 150 rfc for 10 min to pellet the nuclei and retain the small debris in the supernatant.

1.9. Remove supernatant and resuspend nuclei pellet in 100  $\mu$ L Diluted Nuclei Buffer and mix 18  $\mu$ L of nuclei suspension with 2  $\mu$ L of Acridine Orange.

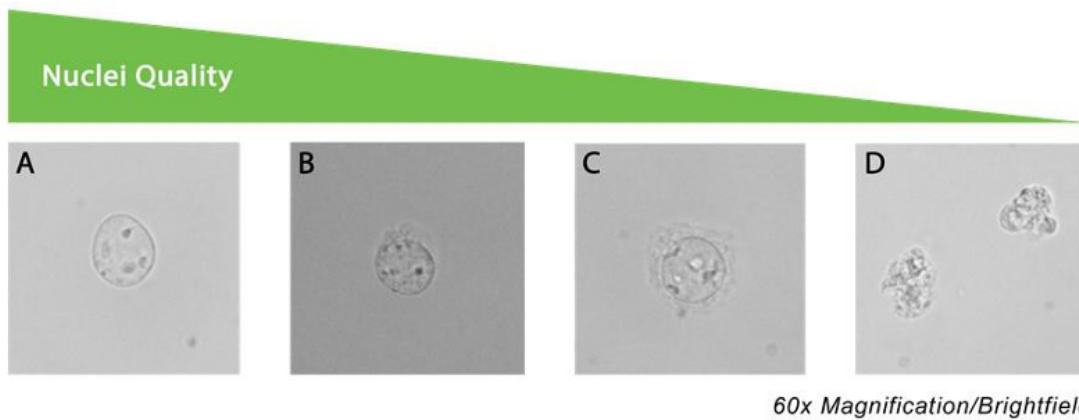


1.10. Count nuclei using a Countess II FL Automated Cell Counter (AOPI staining).



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!

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- 1.12. Centrifuge at 150 rcf for 10 min at 4°C and remove the supernatant without disrupting the nuclei pellet.
  - 1.13. Based on the nuclei concentration estimated at step 1.10, 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.