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Manual Tissue Dissociation for Multiome Analysis

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ABSTRACT

This protocol describes dissociation of snap-frozen ovarian tissue in order to isolate nuclei that can be used for downstream analysis. This protocol can also be used on fresh tissue; freezing tissues leads to a slight decrease in viability after tissue thawing and dissociation. We used this protocol on both ovarian and Fallopian tube snap-frozen tissues. This protocol does not aim to isolate intact ovarian follicles and does not attempt to grossly separate ovarian cortex and medulla. Although this protocol details manual tissue dissociation, we have performed automated tissue dissociation using the S2 Singulator with similar results, for both ovarian and Fallopian tube tissue.

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PROTOCOL CITATION

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MATERIALS TEXT

Wash Buffer: 4mL

- 1% UltraPure BSA, 50 mg/mL (Thermo Fisher Scientific; AM2616)

- Protector RNase Inhibitor, 0.6U/µL (Millipore-Sigma; 3335399001)
- 1X PBS (Gibco; 14200-075)
- *Store at 4°C and keep on ice during use*
- *The buffer will last for up to 5 days, however the RNase Inhibitor should be added the day of use*

Protector RNase Inhibitor **Sigma Aldrich Catalog #03335399001**

UltraPure™ BSA (50 mg/mL) **Thermo Fisher Scientific Catalog # AM2616**

DPBS (10X), no calcium, no magnesium **Thermo Fisher Scientific Catalog #14200075**

NP40 Lysis Buffer: 3mL

- 0.1% Nonidet P40 Substitute (Millipore-Sigma; 74385)
- 10mM Trizma Hydrochloride Solution, pH 7.4 (Millipore-Sigma; T2194)
- 10mM Sodium Chloride Solution, 5M (Millipore-Sigma; 59222C)
- 3mM Magnesium Chloride Solution, 1M (Millipore-Sigma; M1028)
- Nuclease-free water
- *Store at 4°C and keep on ice during use*
- *The buffer will last for up to 5 days*

Nuclease-free Water **Contributed by users**

Nonidet P40 Substitute **Sigma Aldrich Catalog # 74385**

Trizma Hydrochloride Solution pH 7.4 **Sigma Aldrich Catalog #T2194**

Sodium chloride **Sigma Aldrich Catalog #59222C-1000ML**

Magnesium Chloride Solution 1 M **Sigma Aldrich Catalog #M1028**

Protocol:

- Snap-frozen tissue block
- NP40 lysis buffer (see above)
- Ice
- Wash buffer (see above)
- Ice bucket

1.5 mL LoBind tubes **Eppendorf Catalog #022431021**

Trypan Blue Stain (0.4%) for use with the Countess™ Automated Cell Counter **Thermo Fisher Catalog #T10282**

Dry Ice **Contributed by users**

Falcon 40 µm Cell Strainer **Corning Catalog #352340**

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Hemacytometers with Cover Glass
Marienfeld **EF16034F**

Pellet Pestle
Kimble Kontes **K749520-1590**
1.5 mL Polypropylene w/o Microtubes RNAse DNase free

VWR® Pour-Boat Weighing Dishes
flat-bottomed dish
VWR International **10803-166**

BEFORE STARTING

Prepare the Wash Buffer and NP40 Lysis Buffer no more than 5 days in advance.

Methods 22m

1 Keep snap-frozen tissue specimen on dry ice, after removing from freezer and until ready to begin.

2 Using a disposable weigh boat, quickly weigh the tissue specimen while still frozen.

3 Mince tissue with a fresh scalpel until tissue is the size of dry rice grains (or smaller) and transfer to a 1.5mL Eppendorf tube.

4 Add 300µL NP40 lysis buffer. Homogenize with pellet pestle 15X.

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5 Add 1mL NP40 lysis buffer and incubate on ice. The incubation time is tissue dependent. 7m

a. Ovarian tissue: **00:04:00**

b. Fallopian tube tissue: **00:03:00**

6 Strain through a 40µm filter.

7 Centrifuge at 500g, **4 °C** for **00:05:00** . 5m

8 Remove supernatant without disturbing the pellet.

9 Add 1mL wash buffer slowly, **without** mixing.

10 Incubate on ice for **00:05:00** . 5m

11 Slowly pipet to resuspend the pellet.

12 Centrifuge at 500g, **4 °C** for **00:05:00** . 5m

13 Remove supernatant without disturbing the pellet.

14 Add 500µL-1mL wash buffer and pipet to resuspend.

Tip: Additional washes may be necessary based on the quality control checks in step 15 (do not exceed 4 washes).

15 Using Trypan blue, load nuclei onto a hemocytometer and count.

Quality Control: Check under microscope for debris. There are specific acceptable levels of blebbing that we are looking for, specifically levels as represented in images A and B from Panel A in the 10X Genomics document CG000375. We're looking for <5% live cells (ideally no cells), minimal to no clumping, no large debris, minimal to no other debris.

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Tip: Step 14 can be repeated 3 more times if a large amount of debris is still present (do not exceed 4 total washes).

16 Process the isolated nuclei with the 10X Genomics permeabilization protocol described in section 1.2 Nuclei Permeabilization of "Nuclei Isolation from Complex Tissues for Single Cell Multiome ATAC + Gene Expression Sequencing demonstration protocol."

17 Process the permeabilized nuclei with the 10X Genomics Multiomic ATACseq and RNAseq protocols described in "Chromium Next GEM Single Cell Multiome ATAC + Gene Expression Reagent Kits User Guide."

Step-specific processing notes are included below.

17.1 **Step 3.1**: During dynabeads cleanup, aspirate the entire supernatant including the white debris at the bottom.

17.2 **Steps 5.1 and 6.1**: When computing the number of PCR cycles, we round up, when within about 100 nuclei/cells of the threshold for the number of PCR cycles.

17.3 **Step 7.5**: When computing the number of PCR cycles, we do not round up when near the threshold for the number of PCR cycles.

17.4 **Quality control of ATAC library**: While we require the Agilent Bioanalyzer trace at step 5.3 to contain the five expected peaks (0-nucleosome, 1-nucleosome, 2-nucleosomes, 3-nucleosomes, and multiple-nucleosomes), we are somewhat lenient in the expectation that the 1-nucleosome peak will be higher than the other peaks. If peaks are missing, we don't proceed with the sample. If the peaks are visible but the material concentration is low, we recommend rebuilding the library from the cDNA step 4.3.

17.5 **Quality control of cDNA library**: The peak of the Agilent Bioanalyzer trace at step 6.3 should roughly be gaussian centered around 480. If the peak is to narrow or asymmetric, then size selection was not performed correctly and we recommend rebuilding the library from the cDNA step 4.3.

17.6 **Quality control of GEX libraries**: The peak of the Agilent Bioanalyzer trace at step 7.7 should roughly be gaussian centered around 480. If the peak is to narrow or asymmetric, then size selection was not performed correctly and we recommend rebuilding the library from the cDNA step 4.3.

17.7 **Tip**: An Illumina MiSeq run can prove helpful to rebalance the libraries, if needed, and confirm the libraries are properly binding to the flow cell. MiSeq results do not have sufficient sequencing depth for downstream 10X protocol analyses.

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