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Manual Uterine Tissue Dissociation for Multiome Analysis

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Protocol status: Working
This protocol is currently in use by our lab and continues to yield high-quality ATAC-seq and GEX libraries for 10X Genomics Multiome analysis.

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Keywords: ATACseq, RNAseq, Multiome, 10X Genomics, uterus, cervix, single nuclei, manual uterine tissue dissociation for multiome analysis, frozen uterine tissue, frozen uterine tissues in order, manual uterine tissue dissociation, high quality 10x multiome library, cervix tissue biopsy, multiome analysis, human biomolecular atlas program, freezing tissue, using fresh tissue, rna quality, tissue thawing, rna, slight decrease in rna quality, downstream molecular analysis, fresh tissue, tissue

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Abstract

This protocol was developed in accordance with the mission statement of the Human BioMolecular Atlas Program (HuBMAP) of the NIH, to create an open and global platform to map healthy cells in the human body. Protocols developed through HuBMAP can be used by researchers across the world to create Standard Operating Protocols (SOPs) for sample collection and processing.

This protocol describes dissociation of snap-frozen uterine tissues in order to isolate nuclei that can be used for downstream molecular analysis. This protocol can also be used on cell suspensions made from fresh tissue; freezing tissues leads to a slight decrease in RNA quality after tissue thawing and dissociation. If using fresh tissue, we recommend optimizing the lysis time. We use this protocol to generate high quality 10X Multiome libraries from snap-frozen uterine and cervix tissue biopsies.

Materials

Nuclease-free H_2O

- Nonidet P40 Substitute **Merck MilliporeSigma (Sigma-Aldrich) Catalog #** 74385
- Trizma Hydrochloride Solution pH 7.4 **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**T2194
- Sodium chloride **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**59222C-1000ML
- Magnesium chloride solution for molecular biology (1.00 M) **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**M1028
- Tween-20 **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**P-7949
- Protector RNase Inhibitor **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**3335399001
- Ultrapure BSA **Ambion Catalog #**AM2616
- DTT **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**43816-10ML
- Digitonin (5%) **Thermo Fisher Catalog #**BN2006
- Cell strainer 70um filter **Falcon Catalog #**352350
- Bel-Art™ SP Scienceware™ Flowmi™ Cell Strainers for 1000 µL Pipette Tips **Fisher Scientific Catalog #**14-100-150
- Trypan Blue **Invitrogen - Thermo Fisher Catalog #**T10282
- Lance Disposable Scalpel, Sterile Size 22, green handle **Electron Microscopy Sciences Catalog #**72054-48

Ice bucket
Dry ice
Wet ice

Lysis Dilution Buffer
10mM Tris-HLC, pH 7.4
10mM NaCl
3mM $MgCl_2$
1% BSA
1mM DTT
1U/µL Protector RNase Inhibitor
Nuclease-free H_2O

1X Lysis Buffer
10mM Tris-HLC, pH 7.4
10mM NaCl
3mM $MgCl_2$
1% BSA

0.1% Tween-20
0.1% Nonidet NP-40 Substitute
0.01% Digitonin
1mM DTT
1U/µL Protector RNase Inhibitor
Nuclease-free H_2O

0.1X Lysis Buffer
Dilute 1X Lysis buffer in Lysis Dilution Buffer to a final concentration of 0.1X.

Nuclei Wash Buffer
10mM Tris-HLC, pH 7.4
10mM NaCl
3mM $MgCl_2$
1% BSA
0.1% Tween-20
1mM DTT
1U/µL Protector RNase Inhibitor
Nuclease-free H_2O

Diluted Nuclei Buffer 20X Nuclei buffer **10x Genomics Catalog #**2000207
1X Nuclei Buffer (20X Nuclei buffer stock)
1mM DTT
1U/µL Protector RNase Inhibitor
Nuclease-free H_2O

Equipment

C-Chip Disposable Hemocytometer, NI, 100 Tests
NAME

Hemocytometer
TYPE

Bulldog Bio
BRAND

102407-946
SKU

https://us.vwr.com/store/product/13648361/null
LINK

50 slides (2 tests/slide); chamber volume = 10uL
SPECIFICATIONS

Equipment

Falcon™ Plastic Disposable Transfer Pipets
NAME

Transfer pipet
TYPE

Falcon
BRAND

13-680-50
SKU

https://www.fishersci.com/shop/products/corning-plastic-disposable-transfer-pipets-2/1368050?searchHijack=true&searchTerm=357524&searchType=RAPID&matchedCatNo=357524
LINK

3mL volume with graduations
SPECIFICATIONS

Before start

Prepare all buffers fresh on wet ice the day of the experiment. All buffers must be maintained at 4°C.

Prepare a bucket with dry ice.

If using a ceramic mortar, place the mortar on dry ice to pre-chill. The mortar should be ice cold by the time you are ready to mince your sample.

If using a glass Dounce instead of a ceramic mortar, place the glass Dounce on wet ice to pre-chill. The Dounce should be ice-cold by the time you are ready to Dounce your sample.

Methods

59m 50s

1 Retrieve a snap frozen uterus/cervix sample and place it on dry ice. 5m

2 Using a scalpel and the pre-chilled mortar, cut a piece of tissue between 40-150mg, keeping the sample frozen. 1m

3 Using a disposable weigh boat, take the sample weight while keeping the sample frozen. 30s

4 In the pre-chilled mortar, mince the frozen tissue until it's the size of (or a bit smaller) than grains of dry rice. 1m 30s

> Note
>
> If the mortar is properly chilled, the tissue will remain frozen during the mincing process.

5 Using a mechanical tissue disruptor, such as a Drummel, homogenize the minced frozen uterine tissue in 400µL of 0.1X Lysis buffer. 1m

> Note
>
> Adding more than 400µL of Lysis Buffer at this step may cause sample overflow during homogenization.

> Note
>
> In some cases using a mechanical tissue disrupter, such as a Drummel, may produce excessive debris. In this case, you can homogenize the frozen tissue in 400µL 0.1X Lysis Buffer using a pre-chilled ice-cold Dounce homogenizer. When using a Dounce, homogenize tissue using 12-20 stokes with the A (loose) pestle, followed by 15 strokes with the B (tight) pestle.

6 Add 600µL of 0.1X Lysis Buffer to homogenized sample and pipet 2-5x with a 1000µL pipet tip or a wide-bore transfer pipet. 10s

7 Incubate for 5 minutes on ice. 5m

8 Pipette sample 10x using a 1000µL pipet tip or wide-bore transfer pipet. 10s

9 Incubate for 4 minutes on ice. 4m

10 For cervix samples, proceed to Step 11. For uterus samples, proceed to Step 12.

Note

Total lysis time for uterus samples is ~9 minutes.

11 Pipette sample 10x using a 1000µL pipet tip or wide-bore transfer pipet. Incubate for 10 min on ice. 10m

Note

Total lysis time for cervix samples is ~20 minutes.

12 Strain sample through a 70µm cell strainer. The flow-through contains your nuclei suspension. 1m

13 Strain nuclei suspension through a 40µm Flowmi cell strainer. 1m

14 Add an equal volume of Nuclei Wash Buffer and pipette mix 3-5x using a 1000µL pipet tip or wide-bore transfer pipet. 30s

15 Centrifuge at 500 rcf for 5 minutes at 4°C. 5m

16 Discard the supernatant without disrupting the nuclei pellet. 20s

17 Add 500µL Nuclei Wash Buffer and gently pipette 5x to resuspend the nuclei pellet. 1m

18 Centrifuge at 500 rcf for 5 minutes at 4°C. 5m

19 Discard the supernatant without disrupting the nuclei pellet. 20s

20 Repeat Steps 17-19 for a total of 2 washes. 6m 20s

21 Add 500-1000µL Nuclei Wash Buffer. 20s

22 Determine the nuclei count using Trypan Blue exclusion. 5m

Note

Quality control: If significant debris is observed, strain nuclei suspension through a 40µm Flowmi cell strainer; then, determine nuclei concentration using Trypan Blue exclusion.

Note

Quality control: There are acceptable levels of nuclei membrane blebbing, as represented in images A and B from Panel A in the **10X Genomics document CG000375**. An acceptable nuclei suspension will contain <5% live cells (ideally no live cells), minimal to no clumping, minimal to no debris, and no large debris.

23 Centrifuge at 500 rcf for 5 minutes at 4°C. 5m

24 Discard the supernatant without disrupting the nuclei pellet. 20s

25 Resuspend the nuclei pellet in ice-cold Diluted Nuclei Buffer to create your nuclei stock concentration. The volume is calculated based on your nuclei count obtained in step 22 and the appropriate concentration range as determined by your Targeted Nuclei Recovery. 20s

Note

Quality control: for best results, the Targeted Nuclei Recovery should be between 5,000-6,000.

Note

To find the acceptable nuclei stock concentration ranges, refer to the "Nuclei Stock Concentration Table" on page 5 of the 10X Genomics protocol "**Nuclei Isolation from Complex Tissues for Single Cell Multiome ATAC + Gene Expression Sequencing demonstration protocol**."

26 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**."

Protocol references

NIH HuBMAP: **https://hubmapconsortium.org/**

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