Oct 11, 2022

Tissue Dissociation for Multiome Analysis Using S2 Singulator

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dx.doi.org/10.17504/protocols.io.yxmvmndx6g3p/v1

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ABSTRACT

This protocol describes dissociation of snap-frozen tissue in order to isolate nuclei that can be used for downstream analysis. This protocol can also be used on fresh tissue. We used this protocol on snap-frozen cervical (endocervical and ectocervical) tissue and snap-frozen uterine tissue. Unlike the ovary and Fallopian tube, manual dissociation protocols on the cervix and uterine tissue did not yield sufficient quantities of intact nuclei with minimal debris.

DOI

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PROTOCOL CITATION

Stephen Fisher, Marielena Grijalva, Rong Guo, sarahjoh, Hieu Nguyen, John Renz, Jean G Rosario, Steven Rudich, Brian Gregory, Junhyong Kim, Kate O'Neill 2022. Tissue Dissociation for Multiome Analysis Using S2 Singulator. **protocols.io** https://dx.doi.org/10.17504/protocols.io.yxmvmndx6g3p/v1

FUNDERS ACKNOWLEDGEMENT

NIH
Grant ID: U54HD104392

CREATED

Aug 13, 2022

LAST MODIFIED

Oct 11, 2022

PROTOCOL INTEGER ID

68612

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**

Protocol:

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

Nuclei Isolation Kit **S2 Genomics Catalog #100-060-817**

Dry Ice **Contributed by users**

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

Nuclease-free Water **Contributed by users**

- Protector RNase Inhibitor, 0.6U/µL (Millipore-Sigma; 3335399001)

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

Eppendorf Conical Tube 15mL **Merck Millipore Sigma Catalog #EP0030122151**

Singulator 100 System
Tissue dissociation
S2 Genomics **100-067-764**

pluriStrainer® 20 µm (Cell Strainer)
cell strainer
pluriStrainer **43-50020-03**

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

BEFORE STARTING

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

1 Pre-chill nuclei cartridge in the fridge overnight.

Tip: Keep cartridge at **4 °C** until ready for use.

2 Turn the Singulator 100 on by turning on the main machine, tablet, then cooler chamber (in that order).

3 Place the Nuclei Isolation Reagent (NIR) and Nuclei Storage Reagent (NSR) in the cooler and connect them by attaching the correctly labeled luer lock on the machine.

4 If the waste and water bottles are not yet connected, connect those to the machine using the correctly labeled luer locks.

5 Fill water bottle with nuclease-free water.

6 Press “cool” to begin cooling the chamber.

7 While chamber is cooling, quickly weigh sample (optimal sample weight is 25-100mg).

8 Place sample back on dry ice until ready for use.

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9 Set the following parameters for the S2 Genomics “Extended Nuclei Protocol” on the Singulator 100.
a. Auto mince: NO
b. Incubation time: 5 minutes
c. Incubation temperature: COLD
d. Mixing type: TOP
e. Mixing speed: FAST
f. Disruption type: DEFAULT
g. Disruption speed (tissue dependent):
i. Cervix (endocervical and ectocervical) tissue: FASTEST
ii. Uterine tissue: FAST

10 Load nuclei cartridge with sample and 30µL of Protector RNase Inhibitor.

11 Run protocol.

Tip: Watch the camera until the NIR has been dispensed. You may need to jiggle the NIR bottle in the cooling chamber to release the tubing from suctioning the side of the NIR bottle – you should only have to do that once.

12 When protocol has finished running, remove the cartridge from the machine and immediately place on ice.

13 Puncture the aluminum foil seal on the cartridge and remove the nuclei suspension.

14 Pellet suspension at 500g for **00:05:00** at **4 °C** . 5m

15 Remove and discard supernatant.

16 Resuspend nuclei in 1mL wash buffer and place on ice.

Tip: Additional washes may be necessary based on the quality control checks in step 18 (do not exceed 4 washes). Additional clean-ups may also be needed, such as an OptiPrep gradient clean-up as described in “Debris Removal” (Martelotto, 2021).

17 Strain nuclei suspension through a 20µm strainer into a fresh FACS or 15mL conical tube.

18 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 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 16 can be repeated 3 more times if a large amount of debris is still present (do not exceed 4 total washes).

19 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."

Note: Protector RNase Inhibitor concentration is 0.6U/µL.

20 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.

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

20.2 **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.

20.3 **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.

20.4 **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.

20.5 **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.

20.6 **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.

20.7 **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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