May 18, 2021

Tissue Nuclei Isolation and Glutaraldehyde Fixation for sci RNA-Seq

David Fraser Read[1], Cole Trapnell[1]

[1]University of Washington, Dept. of Genome Sciences

In Development Share dx.doi.org/10.17504/protocols.io.budans2e

David Fraser Read

ABSTRACT

This protocol details a method for isolating and fixing nuclei from primary tissue such that the resulting fixed nuclei are compatible with sci RNA-Seq.

A protocol.io page at (https://www.protocols.io/view/sci-rna-seq3-9yih7ue) goes over the sci RNA-Seq workflow. However, the method for nuclei preparation in that protocol does not yield usable nuclei when applied to many primary tissues. The protocol here would replace previous nuclei preparation workflows for some tissues.

DOI

dx.doi.org/10.17504/protocols.io.budans2e

PROTOCOL CITATION

David Fraser Read, Cole Trapnell 2021. Tissue Nuclei Isolation and Glutaraldehyde Fixation for sci RNA-Seq. **protocols.io** https://dx.doi.org/10.17504/protocols.io.budans2e

CREATED

Apr 20, 2021

LAST MODIFIED

May 18, 2021

PROTOCOL INTEGER ID

49282

GUIDELINES

Timeline

Preparing buffers, chilling/labeling tubes, etc. takes ~1 hour.
Dissociation, fixation, and washes take ~1 hour.

Samples to Process

We typically process 2 or 4 samples at a time. More than that is difficult to filter after dissociation while still maintaining a total fixation time of 15 minutes.

Citation: David Fraser Read, Cole Trapnell (05/18/2021). Tissue Nuclei Isolation and Glutaraldehyde Fixation for sci RNA-Seq. https://dx.doi.org/10.17504/protocols.io.budans2e

MATERIALS TEXT

Nuclease free water
Tris HCl (1M, pH 7.4)
Sodium Chloride (5M solution)
Magnesium Chloride (1M solution)
Sodium Phosphate (1M, pH 7.2)
Triton X-100
Polyvinyl Sulfonic Acid (30%, Sigma. Cat 278424)
Vanadyl Ribonucleoside Complex (NEB, S1402S)
Bovine Serum Albumin (NEB, B9000S)
SuperaseIn (Thermo, AM2696)
Diethyl Pyrocarbonate (Sigma, D5758)
Glutaraldehyde (25% solution, Sigma, G5882)
Gentle MACS M Tubes (Miltenyl Biotec, 130-096-335)
70 uM Cell Strainer (Fisher, 22-363-548)
15 mL Falcon Tubes
1.5 mL Eppendorf Tubes

Required Equipment
Gentle MACS Dissociator (Miltenyl Biotec)
Ice buckets
Refrigerated centrifuge with 15 mL tube holders
Liquid nitrogen (for snap-freezing)

SAFETY WARNINGS

The lysis/fixation buffer contains hazardous material (DEPC and glutaraldehyde, in particular). These compounds should be added to the buffer in a chemical safety hood and all work that involves those buffers being open to air should be done in a hood as well. That includes the step adding tissue to MACS tubes, the filtering steps of tissue after MACS dissociation, and so on.

DISCLAIMER:

Buffer Preparaion

1 Nuclei Buffer: 10 mM Tris HCl, 10 mM NaCl, 3 mM MgCl2 in nuclease-free water

Reagent	Stock (mM)	Final Conc. (mM)	Volume (mL)
Tris HCl (pH 7.4)	1000	10	5
NaCl	5000	10	1
MgCl2	1000	3	1.5
Water			492.5

Store at 4C.

4x Hypotonic Phosphate Buffer: 40 mM NaCl, 12 mM MgCl2, 40 mM sodium phosphate (pH 7.).

Citation: David Fraser Read, Cole Trapnell (05/18/2021). Tissue Nuclei Isolation and Glutaraldehyde Fixation for sci RNA-Seq. https://dx.doi.org/10.17504/protocols.io.budans2e

2

Reagent	Stock (mM)	Final Conc. (mM)	Volume (mL)
Sodium Phosphate, pH 7.2	1000	40	8
NaCl	5000	40	1.8
MgCl2	1000	6	2.4
Water			387.8

Store at 4C for up to a month.

Note: Solution precipitates over time, so it is best to prepare relatively small amounts and re-make stocks every couple weeks. Check for white precipitate before use.

3 10% Triton X-100 Stock

Combine 1.0 mL of Triton X-100 with 9.0 mL nuclease-free water. Gently rock overnight to mix, store at 4C.

4 .3% Polyvinyl Sulfonic Acid (PVSA):

Combine 100 uL PVSA (30% stock solution) with 9.9 mL nuclease-free water

5 Nuclei suspension buffer (NSB)

For each sample to be dissociated, prepare 1.2 mL. **Chill on ice**

- 1.2 mL Nuclei Buffer (10 mM Tris HCl pH 7.4, 10 mM NaCl, 3 mM MgCl2)
- 12 uL Bovine Serum Albumin (BSA)
- 12 uL SuperaseIn

Note: 1.2 mL per sample assumes that a single aliquot of fixed nuclei will be frozen and stored per sample. Add 100 uL of volume to this amount for every additional aliquot that you plan to store. (E.g. if planning to split a sample into 3 aliquots to store, make 1.4 mL of buffer for that sample in this step).

6 Lysis/Fixation Buffer:

For each sample to be dissociated, prepare 10 mL of lysis/fixation buffer. **Chill on ice**.

- 2.5 mL of 4x Hypotonic phosphate buffer
- 500 uL Vanadyl Ribonucleoside Complex (200 mM)
- 20 uL of Triton X-100 (10% in water)
- 20 uL PVSA (.3% solution)
- 2 mL glutaraldehyde (25% solution in water)
- 4.9 mL nuclease-free water
- 100 uL diethyl pyrocarbonate (DEPC) **Important: Add immediately before use**

Note: Store ~500 uL aliquots of vanadyl solution frozen at -20C. When thawing for use, thaw completely in 37-42C water bath. If thawed at room temperature, solution has a tendency to stay precipitated upon thaw.

Note: Add DEPC last, and immediately before proceeding to tissue dissociation. DEPC has a short half life and will degrade rapidly if mixed and left sitting. Also, DEPC has a tendency to form an organic layer at the bottom of tubes. Vortex or pipette vigorously to mix into solution, and visually confirm no separation before proceeding.

Dissociation and filtering

For each sample to dissociate, add 5 mL of lysis/fixation buffer (out of 10 mL available per sample) to a Gentle MACS M

Citation: David Fraser Read, Cole Trapnell (05/18/2021). Tissue Nuclei Isolation and Glutaraldehyde Fixation for sci RNA-Seq. https://dx.doi.org/10.17504/protocols.io.budans2e

7 Tube. Store both the M Tube and a second tube (holding the remaining 5 mL of buffer) on ice.

8 Add frozen tissue (~100-250 mg in total, stored on dry ice up until the point) to the MACS M Tube (which already has 5 mL buffer within).

Note: We have used either powdered tissue or small chunks, on the order of <=100 mg per piece.

9 Quickly cap and seal the M Tube holding tissue + buffer. Place into the Gentle MACS, and run the "m_heart_01.01" protocol (a pre-programmed method).

Remove the tube from it's holder, invert once, then re-place the tube and run the cycle again. (In total, the cycle will be run twice).

10 Start timing here for nuclei fixation. The nuclei should be fixed for 15 minutes, but start timing this period when the first dissociation cycle on the Gentle MACS begins.

Note: This is because the filtering in the next step can take longer/shorter depending on number of samples, etc. Do not vary the fixation time as a function of variation in filtering time spent, only fix with respect to (time of dissociation starting + 15 minutes).

11 Place dissociated sample in the MACS tube back on ice. In a chemical safety hood, filter the dissociated tissue solution through a 70 uM strainer. Next, use a new pipette tip to wash the strainer with the 5 mL prepared and stored previously (that was not added to the MACS tube).

12 Incubate on ice for the remainder of the 15 minute period (time begins when the first cycle of dissociation protocol was started). Invert tubes of filtered nuclei every 5 minutes.

13 Centrifuge nuclei. Spin 500 RCF, 8 minutes, at 4C.

Optional: Consider increasing spin time up to 12 minutes for small samples/if particularly high nuclei recovery numbers are key.

14 Remove and dispose of supernatant. Resuspend the pellet gently but thoroughly with 1 mL of NSB (pre-chilled on ice).

15 Centrifuge nuclei. Spin 500 RCF, 5 minutes, at 4C.

16 Remove and dispose of supernatant. Next, resuspend the nuclei gently but thoroughly in the appropriate volume to make up 100 uL aliquots. Resuspend in (10 + (numer-of-aliquots * 100)) uL. So 110 uL if saving 1 aliquot, 210 uL if saving 2 aliquots, 310 if saving 3 aliquots, etc.

Optional: Count nuclei here to guarantee an appropriate number of nuclei are available for later steps. Having <500,000 nuclei in an aliquot makes it difficult to pellet and recover sufficient nuclei later.

17 Disperse 100 uL to separate tubes to snap-freeze as separate aliquots. Snap freeze in liquid nitrogen, store at -80C or colder.

Citation: David Fraser Read, Cole Trapnell (05/18/2021). Tissue Nuclei Isolation and Glutaraldehyde Fixation for sci RNA-Seq. https://dx.doi.org/10.17504/protocols.io.budans2e