PARSE BIOSCIENCES

PARSE DEVELOPED PROTOCOL

Single Nuclei Preparation for Mouse Colon

This protocol was used in

5 Million Mouse Single-Cell Atlas from 7 Tissues

Materials

Material	Supplier	Part Number
Sucrose	Sigma-Aldrich®	S0389
KCl	Invitrogen®	AM9640G
$MgCl_2$	Invitrogen	AM9530g
Tris buffer, pH 8	Invitrogen	AM9856
Nuclease-Free Water	Sigma-Aldrich	W4502
DTT	Invitrogen	P2325
RNase Inhibitor	QIAGEN®	Y9240
SUPERase·In RNase Inhibitor	Invitrogen	AM2696
Triton X-100	Sigma-Aldrich	T8787
DNA low binding, DNase/RNase free, filtered pipette tips 20 µL, 200 µL, 1,000 µL	Varies	Varies
Falcon® High Clarity PP Centrifuge Tubes, 15 mL	Corning®	352097
5.0mL LoBind® Tubes	Eppendorf®	0030108302

Corning 100 mm TC-treated Culture Dish	Corning	430167
RNAse-free razor blade	Varies	Varies
Cell Strainer (40 µm)	Corning	431750
Trypan Blue	Varies	Varies
RNaseZAP® RNase Decontamination Solution	Thermo Fisher Scientific®	AM9780
Ethyl alcohol, Pure	Sigma-Aldrich	459844
Optional: Fluorescent stain (e.g., Hoechst, DAPI, or AOPI)	Varies	Varies
Dounce Tissue Grinder, 1mL	WHEATON®	357538
Single channel pipettes: P20, P200, P1000	Varies	Varies
Cold block	Varies	Varies
RNAse-free forceps	Varies	Varies
Hemocytometer	Sigma-Aldrich	Z359629

Note: In addition to the above materials, you will need access to dry ice for this protocol.

Preparation

1. Clean the bench top and dissection area with 70% ethanol, followed by RNaseZAP to remove RNases.

2. Prepare the following solutions, and add reagents in the order provided in the table.
3. Clean the Dounce homogenizer with RNaseZAP followed by 70% ethanol then rinse well with dH_2O and let dry.

NIM1 buffer

Reagents	Stock Concentration	Final Concentration	Volume for 15 mL
Sucrose	1.5 M	250 mM	2,500 µL
KCl	2 M	25 mM	187.5 µL
$MgCl_2$	1 M	5 mM	75 µL
Tris buffer, pH 8	1 M	10 mM	150 µL
Water	-	-	12,087.5 µL

Homogenization buffer

Reagents	Stock Concentration	Final Concentration	Volume for 5 mL
NIM1 Buffer	1.5 M		4,845 µL
DTT	1 mM	1 µM	5 µL
RNase Inhibitor	40 U/µL	0.4U/µL	50 µL
SUPERase·In RNase Inhibitor	20 U/µL	0.2U/µL	50 µL
Triton X-100	10%	-	50 µL

Note: If there is a potential that your sample was exposed to any external sources of RNases during tissue harvest or if your tissue is known to have elevated RNase activity, you can optionally add in additional RNase inhibitors of your choosing when preparing the homogenization buffer.

Nuclei Extraction

1. Gather a dounce and keep at 4°C until use.
2. Make the NIM1 buffer and keep on ice until use.
3. Make the homogenization buffer and keep on ice until use.
4. Prior to douncing, fill a bucket with dry ice and place a cold block on top of the dry ice to use as a cold surface. Place a culture plate on top of the cold block and wait 5 minutes so the culture plate is sufficiently cooled.
5. Transfer the frozen mouse colon onto the pre-cooled culture plate. Using RNAse-free forceps and a razor blade, cut the elongated colon into 1 cm long sections.
6. Dounce
    a. Add tissue sample to the dounce and resuspend in 700 µL of homogenization buffer.
    b. Perform 5 strokes of loose pestle.
    c. Perform up to 10 - 15 strokes of tight pestle. Add additional strokes of the tight pestle if you still see large pieces.
    d. Add additional homogenization buffer up to 1.4 mL.

7. Filter homogenates with a 40 µm strainer into a 5mL or 15 mL tube.
8. Count the nuclei using a preferred accurate method.

Note: In our experience, nuclei are much stickier than whole cells. For researchers making the single-nuclei suspension for the first time, we suggest confirming that the sample contains mostly single nuclei. To do this, take 10 µL of the sample and stain it with Hoechst, DAPI, or AOPI for more than 5 min, place it on a hemocytometer, and visualize it under a 20x and 40x objective on an epifluorescence microscope. A best practices resource for hemocytometer use, see: https://www.hemocytometer.org/

9. Move up to the maximum number of nuclei* allowed by the Evercode™ Fixation kit into the nuclei fixation workflow.

*Up to 1 million nuclei per reaction for Evercode Nuclei Fixation v3 and up to 100,000 nuclei for Evercode Low Input Nuclei Fixation v3.

