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Protocol status: Working
We use this protocol and it's working

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WU sn-prep Protocol for solid tumors- ATAC v2.7

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ABSTRACT

Single nuclei dissociation protocol for snATAC-seq

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Reagents and Tools

1 1x Lysis buffer (2mL):
10mM Tris-HCl (pH 7.4) (Thermo; 15567027), 20µL
10mM NaCl (Thermo; AM9759), 4µL
3 mM MgCl2 (Thermo; AM9530G), 6µL
NP-40 substitute (Sigma, 74385-1L), 2µL
1 M DTT (Sigma, 646563), 2µL
Nuclease Free Water (Invitrogen, AM9937), 1.966mL

2 Lysis Dilution Buffer (10mL):
10mM Tris-HCl (pH 7.4) (Thermo; 15567027), 100µL
10mM NaCl (Thermo; AM9759), 20µL
3 mM MgCl2 (Thermo; AM9530G), 30µL
1 M DTT (Sigma, 646563), 10µL
Nuclease Free Water (Invitrogen, AM9937), 9.840mL

3 0.1x Lysis Buffer (10mL):
1x Lysis Buffer (1mL) + Lysis Dilution Buffer (9mL)

4 Wash and Resuspension buffer (WR buffer, 10mL):
1X PBS, 8mL
10% Stock BSA Solution (MACS, 130-091-376), 2mL

5 Trypan blue (2X) - filtered at 0.22µm

6 7-AAD (7-Aminoactinomycin D) (Millipore Sigma SML1633-1ML)

7 Glass homogenizers (Fisher: 2mL tube- K8853030002, Small clearance pestle- K8853020002,

Large clearance pestle- K8853010002)

8 Fine forceps and scalpels

General Notes

9
- Keep everything on ice (or in the cold room)
- Avoid foam and bubbles as much as possible with gentle strokes and pipetting slowly
- Use RNase/DNase free reagents and consumables

Nuclei Dissociation

10 If using frozen tissue sample, use a scalpel (aided by a pair of fine forceps) to cut the cold samples (25-35mg) into 2mm pieces, add 1 ml of ice-cold 0.1X lysis buffer, load into the glass homogenizer. Homogenize with 4-6 push/pulls using the pestel, incubate on ice for 1 min with an additional 500-1000µL of 0.1X cold lysis buffer. Pipette gently for 4 times. Incubate on ice again for up to 1 min.

10.1 If using frozen tissue sample, use a scalpel (aided by a pair of fine forceps) to cut the cold samples (25-35mg) into 2mm pieces, add 1 ml of ice-cold 0.1X lysis buffer, load into the glass homogenizer. Homogenize with 4-6 push/pulls using the pestel, incubate on ice for 1 min with an additional 500-1000µL of 0.1X cold lysis buffer. Pipette gently for 4 times. Incubate on ice again for up to 1 min.

10.2 If using OCT sections, start with 300-450µm total sectioned into a 1.5mL tube. Add 1 ml of ice-cold 0.1X lysis buffer, pipette the mix gently for 10-12 times, will be sticky as the OCT thaws. Let sit on ice for 30”. Pipette another 4-6 times with an additional 500-1000µL of 0.1X cold lysis buffer. Incubate on ice again for 1’ – could be reduced (to like 20-45”). Add to the glass homogenizer, dounce with the pestle for 6-8 push/pulls.

11 Filter the homogenate through a 40µM cell strainer on ice on top of a 50ml conical tube. Wash the filter with 1ml WR buffer. Collect this into the same tube, the total filtrate is ~3 ml. If there is still tissue on the strainer, backwash with 2 mL 0.1x Lysis buffer, follow previous steps again to dissociate completely. If going to FACS with the backwash, proceed with this sample as if it were a different tissue but sort into same collection tube.

12 Transfer the filtrate to a 5ml Eppendorf tube. Centrifuge at 500g for 6’ at 4°C, resuspend with

100-400µL WR buffer (depending on pellet size).

13 Transfer into a FACS sorting tube and add 3µL 7-AAD per 500 µL of resuspended sample, incubate for 10 minutes before sorting at FACS. After resuspending sample in wash buffer, if small chunks are still visible, use 40µM mini-strainer over FACS tube to remove chunks (proceeding to FACS with sample in current condition will clog machine and will result in additional lost sample).

14 Add 50µL 10% BSA/PBS solution into a 2mL nonbinding tube for collection. Sort 300-400K of the nuclei into the collection tube.

15 After FACS sorting is done, centrifuge the 2ml collection tube at 500g for 6’ at 4°C. There will not be a visible pellet, remove all the supernatant (likely ~3µL will be left).

16 Resuspend with 3µL 2x Nuclei Dilution Buffer (prepared as directed by 10x Genomics), final volume should be ~10µL.

17 Quantify nuclei quality and quantity using a hemacytometer or Countess II utilizing Trypan blue.

18 Load desired concentration of nuclei, proceed with the protocol as outlined by 10x Genomics.