WU sn-prep Protocol for solid tumors- joint snRNA+ATAC v2.9

Reyka Jayasinghe[1], Wagma Caravan[1], Andrew Houston[1], Nataly Naser Al Deen[1]

[1]Department of Medicine, Washington University in St. Louis, St. Louis, MO 63110, USA

Andrew Houston

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

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ABSTRACT

Nuclei dissociation protocol adapted from WU sn-prep Protocol for Solid Tumors - snRNA protocol v2.8 for simultaneous profiling of genetic expression (snRNA) and chromatin accessibility (snATAC)

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85530

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 Roche Protector RNase inhibitor-2000 U/µL (Millipore Sigma 3335399001)

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

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

8 Glass homogenizers (Fisher: 2mL tube- K8853030002, Small clearance pestle- K8853020002, Large clearance pestle- K8853010002)

9 Fine forceps and scalpels

General Notes

10
- Keep everything on ice (or in the cold room)
- Use RNase free reagents and consumables, before starting wipe down all surfaces/pipettes with RNase away and 70% ethanol
- Avoid foam and bubbles as much as possible with gentle strokes and pipetting slowly

Nuclei Dissociation

11 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 and 30 µL RNase Inhibitor, 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.

11.1 If using pulverized powder, start with 15-35 mg total, add 1 ml of ice-cold 0.1X lysis buffer and 30 µL RNase Inhibitor, pipette the powder/lysis buffer mix gently for 6-8 times. 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, use the pestle for 3-4 push/pulls.

11.2 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 and 30 µL RNase Inhibitor, 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.

12 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.

13 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) and 10 µL RNase inhibitor.

14 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 (after ~3 minutes of resuspension) 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).

15 Add 50µL 10% BSA solution and 10µL RNase inhibitor and into a 2mL nonbinding tube for collection. Sort 300-400K of the nuclei into the collection tube.

16 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).

17 Resuspend in 3µL 2x Nuclei Dilution Buffer (prepared as directed by 10x Genomics, pg. 28) and 1µL RNase inhibitor, final volume should be ~10µL.

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

19 Load desired concentration of nuclei, proceed with the protocol as outlined by 10x Genomics which can be found here.