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WU sn-prep Protocol for Solid Tumors - snRNA protocol v2.8

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Reagents and Tools

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

- 2 Lysis Dilution Buffer (10 mL):
10mM Tris-HCl (pH 7.4) (Thermo; 15567027), 100 uL
10mM NaCl (Thermo; AM9759), 20 uL
3 mM MgCl₂ (Thermo; AM9530G), 30 uL
1 M DTT (Sigma, 646563), 10 uL
Nuclease Free Water (Invitrogen, AM9937), 9.840 mL
- 3 0.1x Lysis Buffer (10 mL):
1x Lysis Buffer (1mL) + Lysis Dilution Buffer (9 mL)
- 4 Wash and Resuspension buffer (WR buffer):
1X PBS, 8 mL
10% Stock BSA Solution (MACS, 130-091-376), 2 mL
- 5 Storage buffer:
1X PBS +2% BSA + 10% glycerol (for freezing) + 0.2U/ul RNase inhibitor
Hard to pipette 100% glycerol. Please make 60% glycerol in 1X PBS. Do a 1:2 mix with the Wash and Resuspension buffer.
- 6 Trypan blue (2X) - filtered at 0.22 mm.
- 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 (Use filtered tips).
 - Avoid foam and bubbles as much as possible by gentle strokes and pipetting.
 - For filtering step, remember to backwash the filter and examine the stuff blocked by the filter if necessary

Single Nucleus Prep for sn-Seq

- 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. Load the pieces into the glass homogenizer. Homogenize by 4-6 pushes and 4-6 pulls using the pestle in a glass homogenizer in 1 ml of ice-cold 0.1X lysis buffer with 30 uL (40 U/uL) RNase Inhibitor. Incubate on ice for 1 min with an additional 1 ml of 0.1X cold lysis buffer. Pipette gently for 4 times. Incubate on ice again for up to 1 min.
- 12 If using pulverized powder, start with 15-35 mg total. Pipette the powder/lysis buffer mix gently for 6-8 times. Let sit on ice for 30". Pipette another 4-6 times. Incubate on ice again for 1' – could be reduced (to like 20-45"). You may choose to homogenize via pestle for 3-4 push/pulls.
- 13 Filter the homogenate through a 40mm cell strainer on ice. Wash the filter with 1ml wash buffer. Collect this into the same filtrate, so the total filtrate is 3 ml. If there is still tissue on the strainer, backwash with 2 mL 0.1x Lysis buffer, and follow previous steps again. If going to FACS with the backwash, proceed with this sample as if it were a different tissue and then sort into same collection tube.
- 14 Transfer this to a 5ml Eppendorf tube. Centrifuge at 500g for 6' at 4°C and resuspend in up to 400ul Wash buffer + ~10 uL RNase inhibitor.
- 15 Transfer into a FACS tube and add 1 uL 7-AAD per 500 uL of sample, and incubate for 10 minutes before FACS. After resuspending sample in wash buffer, if small chunks are still visible (after ~3 minutes of resuspension) use 40 uM 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).
- 16 Add 50 uL WR buffer into a 2 mL nonbinding tube for collection with 10 uL RNase inhibitor. Collect ~100K nuclei into collection tube.
- 17 Once FACS is done, centrifuge at 500g for 6' at 4°C, remove supernatant but leave ~40 uL left in tube. Resuspend in additional WR buffer + RNase inhibitor as needed if too concentrated. Count and check nuclei quality under the microscope and prepare for loading according to the 10X protocol.