Supplementary Protocol 2 – ***Isolation of nuclei from frozen tissues for ATAC-seq***

Protocol Notes

1. All steps should be performed on ice or at 4C. Pre-chill a centrifuge to 4C.
2. It is important to perform the density gradient centrifugation in a swinging bucket centrifuge to avoid collapse of the gradient layers. The original iodixanol gradient protocol calls for a high speed centrifugation (10,000 RCF for 20 minutes). However, such centrifuges are uncommon so we adopted a slower speed centrifugation which still works well. However, higher-speed centrifugation may further improve the purity of nuclei.
3. Make sure to add the correct amount of protease inhibitor tablets to the 6x HB unstable solution
4. See the order list at the end of the protocol for suppliers and catalog numbers

Protocol

1. Place frozen tissue into a pre-chilled 2 ml Dounce with 2 ml cold 1x Homogenization Buffer (HB)
2. Allow frozen tissue to thaw for 5 minutes. Often times the tissue chunk will sink to the bottom of the Dounce once thawed.
3. Dounce with A pestle until resistance goes away (~ 10 strokes). If large chunks of connective tissue are still present, you may need to pre-clear by filtration via a 100 um nylon mesh filter.
4. Dounce with B pestle for 20 strokes
5. Pre-clear larger chunks by pelleting at 100 RCF for 1 min in a pre-chilled centrifuge.
6. Avoiding pelleted chunks of connective tissue, transfer 400 ul to a round bottom 2 ml Lo-Bind eppendorf tube
7. Add 1 volume (400 ul) of 50% Iodixanol solution to give a final concentration of 25% Iodixanol and mix by pipetting
8. Layer 600 ul of 29% Iodixanol solution under the 25% mixture. Avoid mixing of layers.
9. Layer 600 ul of 35% Iodixanol solution under the 29% mixture. Avoid mixing of layers. This step requires gradual removal of the pipette tip during pipetting to avoid excessive volume displacement.
10. In a swinging bucket centrifuge, spin for 20 min at 3,000 RCF with the brake off.
11. Aspirate the top layers down to within 300 ul of the nuclei band
12. Using 200 ul volume, collect the nuclei band and transfer to a fresh tube
13. Count nuclei using Trypan blue staining and aliquot nuclei for ATAC reaction. If the nuclei are too concentrated to accurately count, dilute in ATAC-RSB+0.1% Tween-20
14. Tranfer 50,000 nuclei into a tube containing 1 ml of ATAC-RSB+0.1% Tween-20
15. Centrifuge nuclei for 10 minutes at 500 RCF at 4C
16. Aspirate supernatant and add Omni-ATAC ATAC-seq reaction mix (25 ul 2x TD buffer, 2.5 ul transposase (100nM final), 16.5 ul PBS, 0.5 ul 1% digitonin, 0.5 ul 10% Tween-20, 5 ul H2O) to pellet. Resuspend by pipetting up and down 6 times. No lysis necessary.

Stock Buffer Preparations

Prepare these buffers in advance. These buffers are stable at room temperature. Sterile filtration is recommended, especially for sucrose.

6x Homogenization Buffer Stable Master Mix

Reagent	Final Conc.	Fold Dilution (x)	Vol for 100 mL
1M CaCl2	30 mM	33.33	3 mL
1M Mg(Ac)2	18 mM	55.56	1.8 mL
1M Tris pH 7.8	60 mM	16.67	6 mL
H2O			89.2 mL

1M Sucrose

Add 34.23 g sucrose to 78.5 ml water.

Same Day Buffer Preparations

Prepare these buffers on the day of processing.

6x Homogenization Buffer Unstable Solution (650 ul per sample)

Reagent	Final Conc.	Fold Dilution (x)	Vol per sample
6x Homogenization Buffer Stable	6x	1.00	648.84
100 mM PMSF	0.1 mM	1000.00	1.08
14.3 M β-mercaptoethanol	1 mM	14300.00	0.08

1x Homogenization Buffer Unstable Solution (2 ml per sample)

Reagent	Final Conc.	Fold Dilution (x)	Vol per sample
6x Homogenization Buffer Unstable	1x	6.00	333.33 ul
1M Sucrose	320 mM	3.13	640.00 ul
500 mM EDTA	0.1 mM	5000.00	0.40 ul
10% NP40	0.1%	100.00	20.00 ul
H2O			1006.27 ul

50% Iodixanol Solution (400 ul per sample)

Reagent	Final Conc.	Fold Dilution (x)	Vol per sample
6x Homogenization Buffer Unstable	1x	6.00	66.67 ul
60% Iodixanol Solution	50%	1.20	333.33 ul

29% Iodixanol Solution (600 ul per sample)

Reagent	Final Conc.	Fold Dilution (x)	Vol per sample
6x Homogenization Buffer Unstable	1x	6.00	100.00 ul
1M Sucrose	160 mM	6.25	96.00 ul
60% Iodixanol Solution	29%	2.07	290.00 ul
H2O			114.00 ul

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35% Iodixanol Solution (600 ul per sample)

Reagent	Final Conc.	Fold Dilution (x)	Vol per sample
6x Homogenization Buffer Unstable	1x	6.00	100.00 ul
1M Sucrose	160 mM	6.25	96.00 ul
60% Iodixanol Solution	35%	1.71	350.00 ul
H2O			54.00 ul

Order List

Item	Supplier	Cat Number
Eppendorf 2 ml Lo-Bind tubes	Sigma	Z666556-250EA
Iodixanol (aka Optiprep)	Sigma	D1556-250ML
Sucrose	Sigma	S7903-250G
NP40	Roche (Sigma)	11332473001
EDTA	Ambion (Thermo)	AM9261
β-mercaptoethanol	Sigma	M6250-100ML
PMSF	Sigma	P7626-1G
CaCl2	Sigma	21115-100ML
Mg(Ac)2	Sigma	63052-100ML
Tris pH 7.8	Sigma	T2569-1L
MgCl2	Ambion (Thermo)	AM9530G
KCl	Ambion (Thermo)	AM9640G
H2O	Invitrogen	10977-015
2 ml Dounce Tissue Grinder Set	Sigma	D8938-1SET
150 ml, 0.2 um PES filters	Thermo	124-0045