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Nuclei Isolation from Tissue for 10x Multiome

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HubMAP

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ABSTRACT

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MATERIALS

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

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49520

Stock Buffers

All stock solutions should be filtered using a 0.22 µm PVDF filter system. All solutions except for the 50% Iodixanol solution are stable at 4°C for at least 6 months.

<u>1.034x Homogenization Buffer Stable Solution</u>		For 200 ml stock solution		
<i>Stock</i>	<i>Name</i>	<i>Final Conc.</i>	<i>Fold Dilution (x)</i>	<i>Total Vol. (µl)</i>
1	M Sucrose	0.26	3.87	51706.50
2	M KCl	0.03	77.36	2585.33
1	M MgCl ₂	0.01	193.40	1034.13
0.75	M Tricine-KOH pH 7.8	0.02	36.26	5515.36
-	Water	-	-	139158.69
			Total Vol. (µl)	200000.00

<u>Diluent Buffer</u>		For 100 ml stock solution		
<i>Stock</i>	<i>Name</i>	<i>Final Conc.</i>	<i>Fold Dilution (x)</i>	<i>Total Vol. (µl)</i>
2	M KCl	0.15	13.33	7500.00
1	M MgCl ₂	0.03	33.33	3000.00
0.75	M Tricine-KOH, pH 7.8	0.12	6.25	16000.00
-	Water	-	-	73500.00
			Total Vol. (µl)	100000.00

<u>50% Iodixanol Solution</u>		For 50 ml stock solution		
<i>Stock</i>	<i>Name</i>	<i>Final Conc.</i>	<i>Fold Dilution (x)</i>	<i>Total Vol. (µl)</i>
-	Diluent Buffer	1	-	8333.33
60	% Iodixanol	50	1.20	41666.67
**Remake monthly for stability			Total Vol. (µl)	50000.00

<u>ATAC-RSB Buffer</u>		For 500 ml stock solution		
<i>Stock</i>	<i>Name</i>	<i>Final Conc.</i>	<i>Fold Dilution (x)</i>	<i>Total Vol. (µl)</i>
1	M Tris-HCl pH 7.5	0.01	100.00	5000.00
5	M NaCl	0.01	500.00	1000.00
1	M MgCl ₂	0.003	333.33	1500.00
-	Water	-	-	492500.00
			Total Vol. (µl)	500000.00

<u>1M Sucrose</u>		For 300 ml stock solution		
<i>Stock</i>	<i>Name</i>	<i>Final Conc.</i>	<i>Fold Dilution (x)</i>	<i>Total</i>
-	Sucrose (Powder)	1000	-	102.69 g
	H ₂ O			235.5 ml
			Total Vol. (µl)	300000.00

Same Day Buffers – should be prepared fresh each day

**Note – cOmplete Protease Inhibitors come as tablets. It is difficult to use less than 1/2 tablet so we prepare the 1x Homogenization Buffer Unstable Solution in batches of 12 as outlined below.

<u>1x Homogenization Buffer Unstable Solution</u>				
Stock	Name	Final Conc.	Fold Dilution (x)	Vol per 12 samp. (ul)
1.0341	x HB Stable Solution	1	1.03	24175.00
1	M DTT	0.001	1000.00	25.00
500	mM Spermidine	0.5	1000.00	25.00
150	mMSpermine	0.15	1000.00	25.00
10	% NP40	0.3	33.33	750.00
-	cOmplete Protease Inhibitor	-	-	0.50 Tablets
Total Volume (ul)				25000.00

<u>30% Iodixanol Solution</u>				
Stock	Name	Final Conc.	Fold Dilution (x)	Vol per sample (ul)
-	1x Homog. Buffer Unstable	-	-	240.00
50	% Iodixanol Solution	30	1.67	360.00
Total Volume (ul)				600.00

<u>40% Iodixanol Solution</u>				
Stock	Name	Final Conc.	Fold Dilution (x)	Vol per sample (ul)
-	1x Homog. Buffer Unstable	-	-	120.00
50	% Iodixanol Solution	40	1.25	480.00
Total Volume (ul)				600.00

<u>ATAC-RSB-Tween Buffer</u>				
Stock	Name	Final Conc.	Fold Dilution (x)	Vol per sample (ul)
-	ATAC-RSB	-	-	2970.00
10	% Tween-20	0.1	100.00	30.00
Total Volume (ul)				3000.00

<u>ATAC-seqRxn Mix</u>	
Reagent	Vol per sample (ul)
H2O	5
PBS	16.5
2x TD	25
1% Digitonin	0.5
10% Tween-20	0.5
Tn5	2.5

Order List

Item	Supplier	Cat Number
Eppendorf 2 ml Lo-Bind tubes	Sigma	Z666556-250EA
Eppendorf 1.5 ml Lo-Bind tubes	Sigma	Z666548-250EA
Nunc cryovials	Thermo	375418PK
Iodixanol (comes at 60%)	Sigma	D1556-250ML
Sucrose	Sigma	S7903-250G
NP40	Roche (Sigma)	11332473001
Tricine	Sigma	T0377-25G
Potassium Hydroxide (KOH)	Sigma	P5958-250G
cOmplete Protease Inhibitors	Roche	11697498001
MgCl ₂	Ambion (Thermo)	AM9530G
KCl	Ambion (Thermo)	AM9640G
DTT	Thermo	R0861
Spermidine	Sigma	S2501
Spermine	Sigma	S3256-1G
70 um Flowmi cell strainers	Fisher	03-421-228
70 um bucket-style cell strainers	BD Falcon	352350
Tris-HCl pH 7.5	Invitrogen	15567-027
NaCl	Ambion (Thermo)	AM9759
Tween 20	Roche (Sigma)	11332465001
H ₂ O	Invitrogen	10977-015
Dounce Tissue Grinder Set	Sigma	D8938-1SET
INCYTO Disposable hemocytometers	Fisher	22-600-100
BAM Banker	Wako Chemicals	302-14681
RiboLock	Thermo	EO0384
0.22 um PVDF Filter Units (500 ml)	Millipore	SCGVU05RE
0.22 um PVDF Filter Units (50 ml)	Millipore	SE1M179M6

Before you start the protocol:

- 1) All steps should be performed on ice or at 4°C. Pre-chill a swinging bucket centrifuge and a fixed angle centrifuge to 4°C.
- 2) Pre-chill all Dounces and pestles to 4°C in a fridge.
- 3) Pre-chill all tubes. For each sample you are processing, you will need:
  - a. One 2 ml round-bottom LoBind tube for gradient separation
  - b. One 50 ml conical for filtration step (often optional)
- 4) Prepare all buffers. For faster dissolution, crush protease inhibitor tablets prior to addition to 1x Homogenization Buffer Unstable Solution. DTT, Spermidine, Spermine, and digitonin are stored at -20°C. All other detergents and buffers are stored at 4°C.
  - a. Remember that the catalog number provided for iodixanol from Sigma comes as a 60% solution (not 100%).
- 5) Fill up a 2 L beaker with 500 ml sterile water to soak the used Dounces and pestles.

Isolation of Nuclei via Dounce Homogenization and Density...

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- 1) Remove samples from liquid nitrogen storage and keep on dry ice until use.
- 2) If you are working with a tissue type that is particularly hard to dissociate via Douncing, it can be helpful to pre-crush (do not pulverize) your tissue fragment using mortar and pestle. The tissue must remain frozen and cold during this entire process. Otherwise proceed to Step 3.
- 3) Place 20-50 mg frozen tissue or crushed tissue into a pre-chilled 2 ml Dounce containing 1 ml cold 1x HB (add 15 ul RiboLock per ml of 1x HB and mix well).
- 4) Dounce with "A" loose pestle until resistance goes away (~10 strokes).
- 5) Place "A" pestle into beaker with sterile water to soak for cleaning later.
  - a. Optional – If residual un-homogenized tissue makes it difficult to Dounce, filter homogenate through a pre-chilled 50 ml conical using a 70 um bucket-style cell strainer filter prior to using tight pestle "B".
- 6) Dounce with "B" tight pestle until resistance goes away (~20 strokes).
- 7) Place "B" pestle into beaker with sterile water to soak for cleaning later.
- 8) Filter during transfer using a 40 um cell strainer (Fisher Scientific Cat: 22363547) and transfer homogenate to a pre-chilled 50 ml tube. Transfer homogenate to pre-chilled 2 ml LoBind tube.
- 9) Place Dounce into beaker with sterile water to soak for cleaning later.
- 10) Pellet nuclei by spinning 5 min at 4°C at 350 RCF in a fixed angle centrifuge.
- 11) Remove all supernatant, if the pellet is not clearly visible, you can leave 50 ul supernatant in the tube.
- 12) Gently resuspend nuclei in 350 ul 1x HB, but make sure the total volume of nuclei suspension is 400 ul. Make sure nuclei are fully resuspended without clumps.
- 13) Add 1 volume (400 ul) of 50% Iodixanol Solution and mix well by pipetting
- 14) Slowly layer 600 ul of 30% Iodixanol solution under the 25% mixture. To avoid mixing of layers, wipe the side of the pipette tip with a Kimwipe to remove excess Iodixanol solution from the external surfaces of the pipette tip.
- 15) Layer 600 ul of 40% Iodixanol solution under the 30% mixture. To avoid mixing of layers, wipe the side of the pipette tip with a Kimwipe to remove excess Iodixanol solution from the external surfaces of the pipette tip.
  - a. During this step, you will need to gradually draw your pipette tip up to avoid overflowing the tube. However, the tip of your pipette must stay below the 30%-40% interface at all times.
- 16) In a pre-chilled swinging bucket centrifuge, spin for 20 min at 4°C at 3,000 RCF with the brake off. Handle tubes gently so as to not disturb the gradient.
  - a. Iodixanol is meant to be used at higher speeds (10,000 RCF) but high-speed swinging bucket centrifuges are not always readily available so we perform this step at 3,000 g and have not had any issues.
- 17) Using a vacuum, aspirate the top layers down to within 200-300 ul of the nuclei band at the 30%-40% interface. Be careful not to get too close as you will disrupt the nuclei band.
- 18) Using a 200 ul volume, collect the nuclei band and transfer to a fresh tube. Do not aspirate more than 200 ul at this step as this can cause you to take too much of the 40%

layer which sometimes contains debris.

19) Dilute nuclei by adding 200 μ l of wash buffer (recipe refers to 10x CG000366_DemonstratedProtocol_SingleCellMultiome_Nuclei_EmbMouseBrain__RevB.pdf). Mix gently by pipetting. Filter nuclei suspension to 1.5 ml LoBind tube with 20 μ M Mini-Strainer (PluriSelect Cat: 431002040).

20) Determine the nuclei concentration using a Countess Automated Cell Counter or manual counting using microscope.

21) Centrifuge at 500 rcf for 5 min at 4°C.

22) Base on the nuclei concentration, resuspend nuclei in chilled Diluted Nuclei Buffer (recipe refers to 10x

CG000366_DemonstratedProtocol_SingleCellMultiome_Nuclei_EmbMouseBrain__RevB.pdf

See Nuclei Stock Concentration Table and example Calculation in the User Guide.

Maintain on ice.

23) Proceed immediately to Chromium Next GEM Single Cell Multiome ATAC + Gene Expression User Guide (CG000338)