OmniATAC protocol using Frozen Tissue

Day 1 – Tissue disruption, nuclei isolation, transposition and clean up

Preparations

- Pre-chill a swinging bucket centrifuge 5 mL tubes to 4˚C.
- Pre-chill a fixed rotor centrifuge with capacity for 1.5 mL tubes to 4˚C.
- Pre-heat thermomixer to 68 ˚C.
- Rinse the dounce-homogenizers with (1) milli-Q water, (2) 70 % ethanol and (3) milli-Q water.
- If planning to extract from hard/difficult tissues, prepare mortar or ethanol/dry ice bath.

Tissue disruption and nuclei isolation

Nuclei isolation

1. All steps should be performed **on ice**. A suitable amount of tissue to use can vary, but try starting with 40-60mg.
2. Place frozen tissue into an ice-cold 2 mL dounce-homogenizer containing 1.2 ml cold 1x Homogenization buffer (1xHB).

   NOTES:

   2.1. Allow the frozen tissue to thaw completely in the buffer (max 5 minutes).

   2.2. Preparing hard tissues (e.g. gill), you should try to finely mince into small pieces.

   2.3. The volume of 1xHB buffer can be adjusted up or down to account for using more or less tissue (min = 1 ml, max = 2ml).
3. To release nuclei from cells, dounce with the loose (A) pestle until there is no resistance (~ 5-10 passes depending on tissue type), then dounce with the tight (B) pestle for 5-15 passes.

   NOTES:

   2.1 It is important to control visually the level of homogenization and try not to over homogenize. Note too that different people may apply different strength during homogenization.
4. Pre-clear the solution by filtering it through a 70 um (or 40 um) cell strainer suspended in a 50ml Falcon tube.
5. Transfer 400 ul of filtrated suspension to each of two new 5 mL Eppendorf tube.
6. In each of 5ml tube containing filtrated suspension, prepare a density gradient:

   5.1 Add an equal volume (400 ul) of 50 % iodixanol solution and mix by pipetting (to create a 25% mixture).

   5.2 Aspirate 600 ul of 29 % iodixanol solution into a 1ml pipette tip using Reverse Pipetting technique and wipe the outside of pipette-tip with kimtech paper.

   5.3 Carefully slide the pipette to the base of the 5ml tube, steadily dispense 600ul of 29% iodixanol solution slowly under the 25 % mixture. Slowly withdraw the pipette tip along the side of the tube after dispensing to avoid abrupt volume displacement and mixing of layers.

   5.4 Aspirate 600 ul of 40 % iodixanol solution into a 1ml pipette tip using Reverse Pipetting technique and wipe the outside of pipette-tip with kimtech paper.

5.5 Carefully slide the pipette to the base of the 5ml tube, steadily dispense 600ul of 40% iodixanol solution slowly under the 29 % mixture. Slowly withdraw the pipette tip after dispensing to avoid abrupt volume displacement and mixing of layers.

7. Carefully transfer the 5ml tubes to a pre-cooled swinging bucket centrifuge and centrifuge for 25 min at 4000 RPM (3220g) with brakes set to "off**".**

   NOTE: Density gradient centrifugation is performed in a swinging bucket centrifuge and with brakes off to avoid collapse of the gradient layers. Without brakes, the centrifuge needs an additional 8-9 min to stop. If possible, centrifugation at higher speed is better, as it may improve the purity of nuclei.
8. After centrifugation, the nuclei band should be visible at the interface between 29%-40% iodixanol layers (see Figure 1). From each of two 5ml tubes, carefully aspirate from the top of the liquid column and follow it as it drops to remove the liquid above the nuclei (approximately 1250 ul), do not be greedy and be careful not to aspirate nuclei. Discard the aspirated volume.
9. From each of two 5 ml tubes, collect the nuclei band in a 200ul volume using a p200 pipette and transfer it to a common 1.5ml LoBind Eppendorf tube. You have now a total volume of 400ul of nuclei bands in a single 1.5 ml LoBind tube.

   NOTE: If you cannot see an opaque layer of nuclei band (Fig. 1): using careful pipetting remove and discard the top 1000 ul, then collect 3 successive volumes (from the top layer down) of 200ul into 6 separate 1.5ml LoBind Eppendorf tubes.

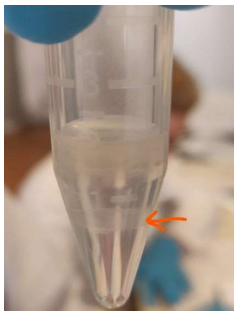


Figure 1. Nuclei band in iodixanol gradient (between 29% and 40%).

10. Add 1000 ul of ATAC-RSB-Tween buffer to the tube containing nuclei and mix by gently inverting.
11. Centrifuge for 10 minutes @ 500 g in a pre-cooled microcentrifuge. Note the orientation of the tube so that you can predict where the pellet should lie.
12. Aspirate supernatant carefully and discard. The nuclei pellet often appears as an opaque "shadow".
13. Resuspend the nuclei pellet in 50 uL of cold PBS.
14. Take an aliquot of 2 ul from nuclei suspension, add 8ul PBS and mix with 10ul of Hoescht. Count nuclei using hemocytometer and assess also the integrity of your sample (shape of nuclei).

    14.1. If there is too much material to accurately count, prepare a new dilution but maintain the ratio of 10ul diluted nuclei suspension (in PBS) : 10ul Hoescht dye.

    14.2. If you couldn't see the nuclei band at Step 8, and collected three 200ul volumes, check each one and select the fraction with the highest concentration of nuclei.

    14.3. If you observe a lot of tissue debris (for example, from muscle), add 100 ul PBS to your nuclei suspension, filtrate through 40um strainer and recount.
15. In a new 1.5ml low-bind tube dilute the appropriate volume of your original nuclei suspension in cold PBS to obtain 16.5ul @ 3000 nuclei/ul (=50,000 nuclei).

Transposase reaction and clean up

Digestion

16. In a new tube, prepare the following transposase reaction mix:

Reagent	Volume per reaction (ul)
2x TD buffer	25
Illumina Transposase	2.5 (100 nM final)
Digitonin (1 %)	0.5
Tween-20 (10 %)	0.5

Nuclease free H_2O	5
Total	33.5

17. Add 33.5 ul transposase reaction mix to 16.5 ul of nuclei suspension and mix by pipetting up and down 6 times.
18. Incubate the reaction at 37 ˚C for 30 minutes in a thermomixer with 1000 RPM mixing. Then, proceed immediately with the next step.

Clean up using MinElute PCR Purification kit from Qiagen

19. Purify the resulting DNA fragments with MinElute PCR purification kit.
19.1. Add **immediately** 5 volumes of PB buffer from MinElute kit, then follow the kit's instructions.
19.2. Elute DNA in 23 ul of EB.
19.3. Measure the DNA concentration with HS Qubit.
20. Eluted DNA can be stored at – 20 ˚C until ready to amplify.

Day 2 –Library amplification

Preparations

- Thaw on ice the transposed DNA, indexes, NEBNext Master Mix and 25X Syber Green mix .
- NOTES: Library amplification is composed of initial PCR amplification (5 cycles) and final PCR amplification (number of additional cycles of each sample to be determined by qPCR). It means that after initial PCR amplification, a small aliquot of amplified libraries is used for qPCR. Initially amplified libraries should be stored at 4˚C until their final amplification. Use PCR tubes for PCR, and qPCR-adapted plates for qPCR.

Initial PCR amplification

21. Set up the following PCR reaction:
21.1. Add 25ul of NEBNext® Ultra™ II Q5® Master Mix to each tube.
21.2. Add a unique combination of Nextera DNA CD index adapters (see Illumina Pooling Guide).
21.3. Add 20 ul of transposed DNA.

Reagent	Volume per sample (ul)
25 uM Nextera DNA CD Index i7	2.5
25 uM Nextera DNA CD Index i5	2.5
NEBNext® Ultra™ II Q5® Master Mix	25
Sample (Transposed DNA)	20
Total	50

22. Short-spin the PCR tubes to collect all the liquid to the bottom.
23. Run the "Initial PCR amplification" program (choose the pre-heat option for lid and set 100˚C)

72 ˚C for 5 min
98 ˚C for 30 sec
98 ˚C for 10 sec
63 ˚C for 30 sec } x5
72 ˚C for 1 min
4 ˚C for forever

Determining the number of final PCR cycles with qPCR

24. Using 5 ul (10 %) of the Initially-amplified product, assemble 15 ul qPCR reactions in a qPCR plate to determine the appropriate number of additional cycles needed.

24.1. Prepare a qPCR mix of nuclease free water, NEBNext® Ultra™ II Q5® Master Mix and 25X SYBRGreen for n+2 samples (n samples + 1 NTC + 1 additional).

Reagent	Volume per sample (ul)
Nuclease free H2O	3.76
NEBNext® Ultra™ II Q5® Master Mix	5
25X SYBRGreen (in H2O*)	0.24
Total	9

** SYBR green is provided at 10,000 X. We make 1ul aliquots and freeze, then on the day of use add 399ul H2O and mix well.*

24.2. Distribute 9 ul of qPCR mix in each well.

24.3. Add a unique combination of Nextera DNA CD index adapters IMPORTANT, this must be the same as was used for Initial PCR reaction.

24.4. Add 5 ul of Initially amplified DNA or 5 ul of water (for NTC).

Reagent	Volume per sample (ul)
25 uM Nextera DNA CD Index i7	0.5
25 uM Nextera DNA CD Index i5	0.5
Initiall amplified DNA	5
Total	6

25. Seal, vortex and centrifuge plate to collect liquid (280g, RT, 1 min).

26. Run the "qPCR" program:

98 ˚C for 30 sec
98 ˚C for 10 sec
63 ˚C for 30 sec
72 ˚C for 1 min
} x20
4 ˚C forever

27. Manually assess the amplification profiles and determine the required number of additional cycles to amplify. The number of cycles should equal ¼ of max fluorescence (Fig. 2).

27.1. For each curve, subtract the baseline value from the end-point value. Then, divide by 4 and check the number of cycles corresponding to that value.

27.2. If the ¼ value is between two cycle numbers, chose the lower one.

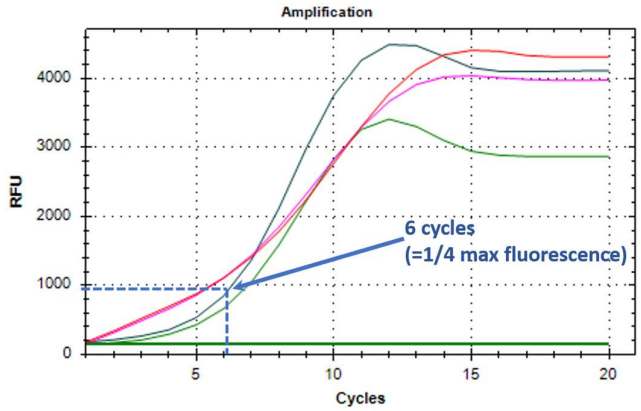


Figure 2. qPCR amplification plot showing ¼ of max fluorescence = 6 PCR cycles

Final PCR amplification

28. Short-spin the PCR tubes containing your Initially amplified product (45ul).
29. Run the "Final PCR amplification" program on your Initially amplified product (45ul) without adding anything:

29.1. Chose the pre-heat option for lid and set 100 ˚C

98 ˚C for 30 sec
98 ˚C for 10 sec
63 ˚C for 30 sec
72 ˚C for 1 min
} x number of cycles determined individually for each sample by qPCR
4 ˚C for forever

Notes: After Final PCR amplification, keep PCR product at 4˚C until you proceed with size selection. It is possible to assess the profile of your libraries on Bioanalyzer before size selection, as you could already see the characteristic peaks. To assess the BA profile, use first High sensitivity Qubit kit to measure the concentration, then dilute a 1 ul aliquot of your PCR product before loading to BA (use High Sensitivity kit).

Day 2 – Library size selection

Preparations

- You will need:
  - Magnet (for Eppendorf tubes)
  - Freshly prepared 80 % ethanol
  - Room temperature Ampure XP beads (Beckman Coulter, Cat# A63881)
  - LoBind Eppendorf tubes
  - Room temperature TET Buffer

Library size selection

Remove small fragments and fragments above 670 bp

1. Thoroughly resuspend Ampure XP beads by vortexing for at least 1 min.
2. Add 0.53x volume (23.9 ul if you have 45ul) of beads to sample (this will preferentially bind to longer DNA fragments).
3. Mix well by pipetting but be gentle to avoid bubbles.
4. Incubate at room temperature for 10 min, while mixing gently every 5 minutes.
5. Touch spin to collect liquid, then place tube on magnetic rack and allow to stand for 5 min (until the supernatant is clear of beads).
6. **Transfer the supernatant** to a new tube and another 1.3x original volume (58.5 ul if you had 45ul) Ampure beads to the supernatant.
7. Mix well by pipetting but be gentle to avoid bubbles.
8. Incubate at room temperature for 15 min while mixing gently every 5 minutes.
9. Place on magnetic rack and let it stand for 5 min (until the supernatant is clear of beads).
10. Remove and **discard supernatant**.
11. While the tubes are still on the magnet, and without disturbing the pellet, wash beads with 100ul of freshly prepared 80% ethanol, remove ethanol and discard. Repeat once.
12. Remove the tubes from the magnet, touch spin the samples, place back on the magnet and carefully remove the last traces of ethanol.
13. Remove samples from the magnet and allow the tubes to air dry (between 30 sec-2min, don't wait until you see small cracks in the beads!).
14. Add 16.5ul TET Buffer. Resuspend beads by pipetting.
15. Rehydrate at room temperature for a minimum of 2 minutes.
16. Place on magnetic rack and let it stand for 5 min (until the supernatant is clear of beads).
17. Transfer the supernatant (eluted DNA) to a LoBind Eppendorf tube or 96 well plate.

18. Measure DNA library concentration with Qubit High Sensitivity Kit.

19. Validate DNA fragment size distribution with Bioanalyzer High Sensitivity DNA kit.

19.1. The DNA fragment size distribution should follow a nucleosome pattern (Fig. 3)

19.1.1. If you have an excess of primer-dimers in your samples, do an addition clean up with MinElute PCR purification column and elute with 10 ul.

19.1.2. If you still have an excess of long fragments in your samples (>1000 bp), do an additional round of bead purification.

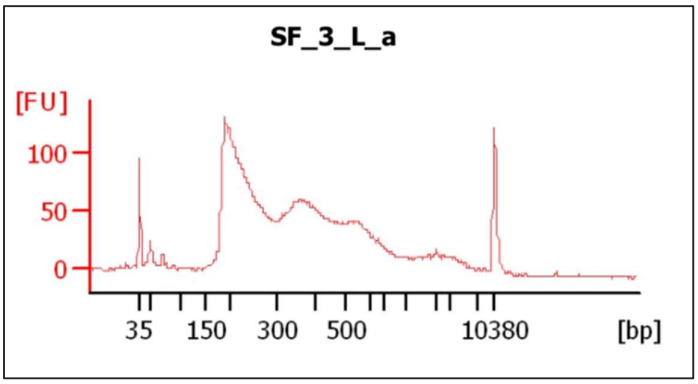


Figure 3. Example of bioanalyzer trace after library preparation and clean up.

Buffers:

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 (store at RT)	**Final conc.**	**Vol for 100 mL**
1 M CaCl2	30 mM	3 mL
1 M Mg(Ac)2	18 mM	1.8 mL
1 M Tris pH 7.8	60 mM	6 mL
dH2O		89.2 mL

1 M Sucrose (store at 4°C)	
Add 34.23 g sucrose to 78.5 mL water	

ATAC-RSB (store at 4°C)	**Final conc.**	**Vol for 50 mL**
1 M Tris pH 7.4	10 mM	500 ul
5 M NaCl	10 mM	100 ul
1 M MgCl2	3 mM	150 ul
dH2O		49.25 mL

PBS (store at 4°C)
Dissolve one tablet of PBS in 200 mL of Milli-Q water

Detergents
Digitonin *is supplied at 2% in DMSO. Dilute 1:1 with water to make a 1% (100x) stock solution. Avoid more than 5 freeze thaw cycles. Can be kept at -20°C for up to 6 months.*
Tween-20 *is supplied at 10%. Use at this concentration (100x stock). Store at 4°C*
NP40 *is supplied at 10%. Use at this concentration (100x stock). Store at 4°C*

Iodixanol 60%
Iodixanol (60 %) OptiPrep *should be kept away from direct sunlight (wrap in aluminium foil), shake the bottle prior to use. Store at RT.*

TET buffer (16.5 ul per sample) (store at 4°C)	**Final conc.**	**Vol per sample (ul)**	**Vol for 100 samples**
Tris-HCL pH 8.0 (1 M)	10 mM	0,165	16,5
EDTA (0.5 M)	1 mM	0,033	3,3
Tween-20 (10 %)	0,05 %	0,0825	8,25
dH2O		16,2195	1621,95

Same day buffer preparations, prepare solutions in fume hood. Keep everything on ice.

	ENTER NUMBER OF SAMPLES	3

12x PIC solution	**Final conc.**	**Vol per sample (ul)**	**Vol total (ul)**
6x Homogenisation Buffer Stable Master Mix		833	1666
PIC tablet	12x	1	2
		TOTAL	1666,00

6x Homogenization Buffer Unstable Solution (910 ul per sample)	**Final conc.**	**Vol per sample (ul)**	**Vol total (ul)**
12x PIC solution	6x	454,19	1362,56
6x Homogenization Buffer Stable Master Mix	6x	454,19	1362,56
100 mM PMSF	0.1 mM	1,51	4,54
14.3 M beta-mercaptoethanol	1 mM	0,11	0,34
		TOTAL	2730

1x Homogenization Buffer Unstable Solution (max 2 mL per sample)	**Final conc.**	**Vol per sample (ul)**	**Vol total (ul)**
6x Homogenization Buffer Unstable	1x	333,33	999,99
1 M Sucrose	320 mM	640,00	1920,00
500 mM EDTA	0.1 mM	0,40	1,20
10 % NP40	0,30 %	60,00	180,00
1 M Spermidine	0.5 mM	1,00	3,00
150 mM Spermine	0.15 mM	2,00	6
dH2O		963,27	2889,81
		TOTAL	6000,00

50 % Iodixanol Solution (800 ul per sample)	**Final conc.**	**Vol per sample (ul)**	**Vol total (ul)**
6x Homogenization Buffer Unstable	1x	133,34	400,02
60 % Iodixanol Solution (OptiPrep)	50 %	666,66	1999,98
		TOTAL	2400

29 % Iodixanol Solution (1200 ul per sample)	**Final conc.**	**Vol per sample (ul)**	**Vol total (ul)**
6x Homogenization Buffer Unstable	1x	200,00	600,00
1 M Sucrose	160 mM	192,00	576,00
60 % Iodixanol Solution	29 %	580,00	1740,00
dH2O		228,00	684,00
		TOTAL	3600,00

40 % Iodixanol Solution (1200 ul per sample)	**Final conc.**	**Vol per sample (ul)**	**Vol total (ul)**
6x Homogenization Buffer Unstable	1x	200,00	600,00
1 M Sucrose	160 mM	192,00	576,00
60 % Iodixanol Solution	35 %	800,00	2400,00
dH2O		8,00	24,00
		TOTAL	3600,00

ATAC-RSB-Tween 20 (1000 ul per sample)	**Final conc.**	**Vol per sample (ul)**	**Vol total (ul)**
ATAC-RSB	1x	990,00	2970,00
10% Tween-20	0.1%	10,00	30,00
		TOTAL	3000,00

OmniATAC protocol using Frozen Tissue (tissue specific modifications)

Liver and brain

Follow the original protocol. Recommended weight 40-50mg for liver and 20-30mg for brain. No filtering needed.

Muscle

Requirement for the amount of tissue is larger than for any other tissues. Start with minimum 100mg. Larger amount of tissue also requires a larger volume of homogenization buffer (1xHB). Start with minimum 1.6mL of 1xHB.
Before homogenization of tissue with pestles, grind the tissue on mortar placed on dry ice until you obtain powder. Filter through 70um cell strainer or through 40um if many debris persist.

Gill

Use 30-40mg tissue. Filter through 40um strainer. When counting prior to transpositions, you should easily see clean, well homogenized and intact nuclei. However, gill nuclei seem harder to lyse and it requires specific conditions of transposition reaction. Use 25k nuclei per reaction and use twice the amount of digitonin (1ul) and Tween-20 (1ul), adjust the volume of water (4ul instead of 5ul) for a total volume of final 50ul of reaction mix.

Head kidney

Add Heparin to 1xHB for a final concentration of 10 units/mL. Then follow the homogenization step with a recommended weight of 20-30mg. Small amount of tissue yields high number of clean nuclei. After the extraction of nuclei band from iodixanol gradient centrifugation, **DO NOT** add the ATAC-RSB or PBS (heavy nuclei aggregation when in direct contact with these buffers). Instead, aspirate a volume of nuclei band corresponding to 25k nuclei (ideally corresponding to 1-2 ul) and add it to 48-49ul of the transposase mix (containing 33.5ul of pre-mix **and** 14.5-15.5ul of PBS).
Use 2.5ul of transposase enzyme for 25k nuclei.

Gonad

Gonad **immature**: follow the original protocol.
Gonad **mature female:** not tested (follow the original male mature-specific protocol or immature gonad protocol).
Gonad **male mature**: Requires multiple washing steps prior to tissue homogenization in order to remove the sperm. Also, in order to remove the maximum of sperm and improve the ratio of sperm: tissue nuclei, and an additional iodixanol gradient centrifugation is needed. This second gradient centrifugation is performed in 2mL tubes that fits microcentrifuges and therefore a higher g force is applied.

Nuclei isolation

30. Buffer preparation for mature male gonad needs larger volumes of 1xHB (9mL/sample more than usual, due to multiple washing steps and second iodixanol centrifugation) and of 50% iodixanol solution (500ul/sample more than usual, due to second iodixanol centrifugation step). All steps should be performed **on ice**. A suitable weight of male mature gonad is 150-200mg.

WASHING

31. Place the tissue in a 5mL tube and add 1mL of 1x Homogenization buffer (1xHB). Gently squeeze the tissue with a 1000ul pipette tip (or with plastic pestle) against the tube side in order to release the sperm but do not disrupt the tissue into smaller pieces.
32. Vortex for 30 seconds minimum and filtrate through 40um cell strainer suspended in a 50mL Falcon tube. Add 200ul of 1xHB over the cell strainer to rinse the tissue and place the tissue back to the 5 mL tube. Discard the filtrate containing mostly sperm.
33. Repeat the step 2 & 3 seven times or until the solution containing the tissue is clear.

TISSUE HOMOGENISATION

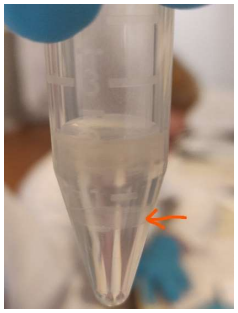
34. Cut the tissue in pieces and place it into an ice-cold 2 mL dounce-homogenizer containing 1 ml cold 1xHB.
35. To release nuclei from cells, dounce with the loose (A) pestle until there is no resistance (~ 5 passes), then dounce with the tight (B) pestle for 2 passes.
NOTES:
7.1 It is important to control visually the level of homogenization and try not to over homogenize. Note too that different people may apply different strength during homogenization. You can also use scalpel to cut the tissue into smaller chunks prior to homogenization.
36. Pre-clear the solution by filtering it through a 70 um cell strainer suspended in a 50ml Falcon tube.

FIRST GRADIENT CENTRIFUGATION

37. Transfer 400 ul of filtrated suspension to each of two new 5 mL Eppendorf tube.
38. In each of 5ml tube containing filtrated suspension, prepare a density gradient:
5.2 Add an equal volume (400 ul) of 50 % iodixanol solution and mix by pipetting (to create a 25% mixture).
5.2 Aspirate 600 ul of 29 % iodixanol solution into a 1ml pipette tip using Reverse Pipetting technique and wipe the outside of pipette-tip with kimtech paper.
5.3 Carefully slide the pipette to the base of the 5ml tube, steadily dispense 600ul of 29% iodixanol solution slowly under the 25 % mixture. Slowly withdraw the pipette tip along the side of the tube after dispensing to avoid abrupt volume displacement and mixing of layers.
5.4 Aspirate 600 ul of 40 % iodixanol solution into a 1ml pipette tip using Reverse Pipetting technique and wipe the outside of pipette-tip with kimtech paper.
5.6 Carefully slide the pipette to the base of the 5ml tube, steadily dispense 600ul of 40% iodixanol solution slowly under the 29 % mixture. Slowly withdraw the pipette tip after dispensing to avoid abrupt volume displacement and mixing of layers.
39. Carefully transfer the 5ml tubes to a pre-cooled swinging bucket centrifuge and centrifuge for 30 min at 4000 RPM (3220g) with brakes set to "off**".**
NOTE: Density gradient centrifugation is performed in a swinging bucket centrifuge and with brakes off to avoid collapse of the gradient layers. Without brakes, the centrifuge needs an additional 8-9 min to stop. If possible, centrifugation at higher speed is better, as it may improve the purity of nuclei.

40. After centrifugation, the nuclei band should be visible at the interface between 29%-40% iodixanol layers (see Figure 1). From each of two 5ml tubes, carefully aspirate from the top of the liquid column and follow it as it drops to remove the liquid above the nuclei (approximately 1250 ul), do not be greedy and be careful not to aspirate nuclei. Discard the aspirated volume.
41. From each of two 5 ml tubes, collect the nuclei band in a 200ul volume using a p200 pipette and transfer it to a common 2ml LoBind Eppendorf tube. You have now a total volume of 400ul of nuclei bands in a single 2 ml LoBind tube.

Figure 1. Nuclei band in iodixanol gradient (between 29% and 40%).



SECOND GRADIENT CENTRIFUGATION

42. Dilute your 400ul of nuclei band by adding the equal volume of 1xHB. Your nuclei band being initially within a 29-40% iodixanol suspension is now diluted to 15-20% iodixanol suspension. Aspirate 500ul of 50% iodixanol solution and carefully slide the pipette to the base of the 2ml tube, then steadily dispense it under the 15-20 % mixture.
43. Carefully transfer the 2ml tube to a pre-cooled microcentrifuge and centrifuge for 40 min @ 12000g with soft stop "on**" if possible.**
44. Collect the nuclei band (around 100-150ul) found between the top (15-20% iodixanol) and bottom (50% iodixanol) layer. **Do not** collect the pellet found at the bottom of the tube, containing mostly sperm.
45. Add 1000 ul of ATAC-RSB-Tween buffer to the tube containing nuclei and mix by gently inverting.
46. Centrifuge for 10 minutes @ 500 g in a pre-cooled microcentrifuge. Note the orientation of the tube so that you can predict where the pellet should lie.
47. Aspirate supernatant carefully and discard. The nuclei pellet often appears as an opaque "shadow".
48. Resuspend the nuclei pellet in 50 uL of cold PBS.
49. Take an aliquot of 2 ul from nuclei suspension, add 8ul PBS and mix with 10ul of Hoescht. Count nuclei using hemocytometer and assess also the integrity of your sample (shape of nuclei).
    49.1. If there is too much material to accurately count, prepare a new dilution but maintain the ratio of 10ul diluted nuclei suspension (in PBS) : 10ul Hoescht dye.
    17.2 If you observe a lot of tissue debris (for example, from muscle), add 100 ul PBS to your nuclei suspension, filtrate through 40um strainer and recount.
50. In a new 1.5ml low-bind tube dilute the appropriate volume of your original nuclei suspension in cold PBS to obtain 16.5ul @ 3000 nuclei/ul (=50,000 nuclei).

Transposase reaction and clean up: **FOLLOW THE ORIGINAL PROTOCOL**

Purchase list

Reagents		
Product	**Company**	**Reference**
Tris-HCl 1M pH 8	Sigma-Aldrich	T2694-100ML
Tris-HCl 1M pH 7.8	Sigma-Aldrich	T2569-100ML
Tris-HCL 1M pH 7.4	Sigma-Aldrich	T2194-100ML
EDTA 0.5M pH 8.0	Sigma-Aldrich	03690-100ML
NaCl (prepare 5M solution)	Sigma-Aldrich	S3014-500G
PBS tablets	Sigma-Aldrich	P4417-50TAB
Sucrose (prepare 1 M solution)	Sigma-Aldrich	84097
CaCl2 (prepare 1 M solution)	Sigma-Aldrich	C5670
Tween-20	Sigma-Aldrich	11332465001
PMSF 0.1M	Sigma-Aldrich	93482-50ML-F
MgCl2 1M	Sigma-Aldrich	M1028-100ML
Mg(Ac)2 1M	Sigma-Aldrich	63052
Digitonin (dilute 1:1 with water to make a 1% (100x) stock solution)	Promega	G9441
NP40	Sigma-Aldrich	11332473001
B-mercaptoethanol	Sigma-Aldrich	M6250
OptiPrep	Sigma-Aldrich	D1556-250ML
Spermidine (make 1 M stock and aliquot in at -20)	Sigma-Aldrich	S2626
Spermine (make 1 M stock and aliquot at -20)	Sigma-Aldrich	S3256
cOmplete, Mini, EDTA-free Protease Inhibitor Cocktail	Sigma-Aldrich	4693159001
Illumina Tagment DNA Enzyme and Buffer	Illumina	20034197
NEBNext® Ultra™ II Q5® Master Mix	NEB	M0544S
Nextera™ DNA CD Indexes (24 Indexes, 24 Samples)	Illumina	20018707
MinElute PCR purification kit	Qiagen	28004
Agencourt Ampure XP beads	Beckman Coulter	A63880
Bioanalyzer High Sensitivity kit and DNA Chips	Agilent Technologies	5067-4626
Qubit High Sensitivity kit	ThermoFisher	Q32854
Qubit assays tubes	ThermoFisher	Q32856
Hoescht dye	ThermoFisher	62249
SYBR™ Green I Nucleic Acid Gel Stain, 10,000X concentrate in DMSO	Invitrogen	S7563
Heparin 180USP/mg (make a 4000 U/ml sotck, store in the fridge)	Sigma-Aldrich	H3393
IDT for Illumina Nextera DNA UD Indexes	Illumina	20027213-20027216

Other material		
Product	Company	Reference
Tissue Grind Tube for 2 mL Tissue Grinder (douncer)	VWR	KT885303-0002
Large Clearance Pestle for 2 mL Tissue Grinder (pestle A)	VWR	KT885301-0002
Small Clearance Pestle for 2 mL Tissue Grinder (pestle B)	VWR	KT885302-0002
Swinging bucket centrifuge for 5 mL tubes with cooling option	Eppendorf	5810R
5 mL Eppendorf Tube snap cap	VWR	89429-306
40 um cell strainer	Corning	431750
70 um cell strainer	Corning	431751
1.5 LoBind Eppendorf Tube	Eppendorf	30120,086
Fixed angle centrifuge for 1.5-2 mL tubes with cooling option	Beckman Coulter	B31607
Thermomixer	Eppendorf	5382000015
Microscope	Zeiss	AX10
Counting chamber BLAUBRAND® Thoma pattern	Brand/VWR	BRND718005
qPCR machine and equipment	BioRad	
Sarstedt Microtest 96 well plate, conical bottom	Sarstedt	82,1583
Magnetic rack	Life Technologies	12321D
PCR plates	VWR	7322390
Plate seal for cover only	Thermo Scientific	AB0580
PCR film for high protection	Thermo Scientific	AB0558
Bioanalyzer/Tape station	Agilent	
Qubit 4 Fluorometer	Invitrogen	Q33226
Dry ice		
Mortar and pestle		