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

1. 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

2. 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.
3. 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.
4. Repeat the step 2 & 3 seven times or until the solution containing the tissue is clear.

TISSUE HOMOGENISATION

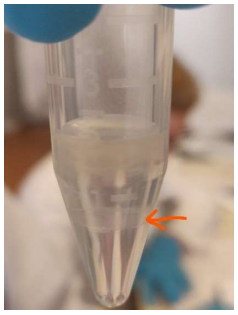
5. Cut the tissue in pieces and place it into an ice-cold 2 mL dounce-homogenizer containing 1 ml cold 1xHB.
6. To release nuclei from cells, dounce with the loose (A) pestle until there is no resistance (~ 5 passes), then dounce with the tight (B) pestle for 2 passes.
   NOTES:
   7.1 It is important 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.
7. Pre-clear the solution by filtering it through a 70 um cell strainer suspended in a 50ml Falcon tube.

FIRST GRADIENT CENTRIFUGATION

8. Transfer 400 ul of filtrated suspension to each of two new 5 mL Eppendorf tube.
9. 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.
10. 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.
11. 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.

12. 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

13. 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.
14. Carefully transfer the 2ml tube to a pre-cooled microcentrifuge and centrifuge for 40 min @ 12000g with soft stop "on**" if possible.**
15. 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.
16. Add 1000 ul of ATAC-RSB-Tween buffer to the tube containing nuclei and mix by gently inverting.
17. 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.
18. Aspirate supernatant carefully and discard. The nuclei pellet often appears as an opaque "shadow".
19. Resuspend the nuclei pellet in 50 uL of cold PBS.
20. 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).
    20.1. 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.
21. 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**