OmniATAC protocol using Frozen Tissue

Overview:
Both for ATAC and ChIP libraries, the protocols for the tissues were slightly altered from the original AquaFAANG protocols to better serve their implementation in gilthead seabream's tissues. The changes in ATAC protocol concern mostly the process of the tissue disruption and nuclei isolation. For different tissues, different changes have been made. All steps were performed on ice.
All the buffers and solutions were prepared according to the original AquaFAANG instructions.

Tissue disruption and nuclei isolation

A suitable amount of each tissue (**Table 1Σφάλμα! Το αρχείο προέλευσης της αναφοράς δεν βρέθηκε.**) was weighed (while still frozen),and placed into an ice-cold dounce-homogenizer containing 1.2 ml cold 1x Homogenization buffer where it was left to thaw completely in the buffer.
1. Most tissues were dounched with the loose (A) and tight (B) pestle gently until there was no resistance (**Table 1**). Some tissues received additional grinding apart from dounche-homogenizer (**Table 1**).
2. The solution was filtered through a 70um cell strainer (or 40um or 100um (**Table 1**))in a 50 ml eppendorf tube
3. 400ul of filtrated suspension was transferred to each of two 5 ml eppendorf tubes.
4. In each of 5ml tube containing filtrated suspension, a density gradient was prepared (without the reverse pipetting method):
    4.1 Add an equal volume (400 ul) of 50 % iodixanol solution and mix by pipetting (to create a 25% mixture).
    4.2 Aspirate 600 ul of 29 % iodixanol solution into a 1ml pipette tip and wipe the outside of pipette-tip with kimtech paper.
    4.3 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.
    4.4 Aspirate 600 ul of 40 % iodixanol solution into a 1ml pipette tip and wipe the outside of pipette-tip with kimtech paper.
    4.5 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.
5. The 5 ml tubes were carefully transferred into a pre-cooled, fixed rotor centrifuge and centrifuged for 25 min at 4000 RPM with brakes set to "off.
6. After centrifugation the nuclei band that was visible at the interface between 29%-40% iodixanol layers was carefully collected in a 200ul volume using a p200 pipette. The bands from both 5 ml tubes were collected and joined in a 1.5ml tube (400ul volume).
7. 1000 ul of ATAC-RSB-Tween buffer were added to the tube containing nuclei and mixed by gently inverting.
8. The sample was centrifuged in a fixed-rotor centrifuge (10 ', 4^{o}C, 500g)., and the supernatant was removed carefully, without disturbing the nuclei pellet.
9. The nuclei pellet was re-suspended in 50ul cold PBS.

10. An aliquot of 2 ul from the nuclei suspension was used in order to count the nuclei and observe their quality. 2 ul of nuclei were mixed with 8 ul PBS and 10ul trypan blue 0.4%. The mix was left for 5' before it was loaded in a hemocytometer and observed.
11. After measuring the concentration of nuclei, a new dilution was made in a new 1.5ml eppendorf tube, where 50000-60000 nuclei (25000 nuclei for gills) where obtained in total volume of 16.5ul.

Table 1:Treatment of different tissues during homogenization and nuclei isolation

Tissue type	mg of tissue	Homogenization
Liver	50-60	1-2 passes pestle A, 2-5 passes pestle B in Dounche Homogenizer
Brain	30-60	1-2 passes pestle A, 2-5 passes pestle B in Dounche Homogenizer
Gills	60-80	The tissue was quickly washed in dH2O to remove any excess blood. Then it was cut I small pieces in a petri dish with 700 ul HB buffer 1x (on ice). The tissue pieces with the liquid where transferred in a dounche homogenizer, where additional 500 ul HB buffer 1x was added. The tissue was dounched with 2-3 passes with pestle A and 2-3 passes with pestle B. Then the solution was filtered through 40um cell strainer.
Muscle	150-200	The tissue was ground in a mortar which was kept on dry ice, until powder was obtained. The powder was transferred to a 1.5 ml eppendorf tube tube which contained 1 ml HB buffer 1x. The solution was mixed by pipetting (cut 1ml tip). The solution was filtered through 70um cell strainer and the filter was washed with 200ul 1xHB. The flow through was transferred in a dounche homogenizer, where it was treated with 2 passes with pestle B.
Mature ♂Gonad	200-270	For this tissue the original tissue specific protocol by AquaFAANG was followed. The only difference is that the gradients were not done with the reverse pipetting technique
Mature ♀Gonad	70	The eggs were removed from the tissue by washing/mixing the tissue in dH2O and manually with the help of watchmaker forceps. The tissue was cut in small pieces in a petri dish with 1.2ml HB buffer 1x (on ice). Then it was homogenized by pipetting up-down with 1000x pippette (cut tip). Then the samples were immediately filtered through 70um cell strainer without using the dounche homogenizer.
Immature ♂Gonad	5	The tissue was cut in small pieces in a petri dish with 600 ul HB buffer 1x (on ice). The tissue pieces with the liquid where transferred to a 1.5 ml eppendorf tube and mixed by pipetting (cut 1ml tip). Then the sample was transferred in a dounche homogenizer, there it was homogenized with 1 pass with pestle A and 2-3 passes with pestle B. The solution was filtered in 100um cell strainer. In step 3, 400ul of filtrated suspension were transferred only in one 5 ml eppendorf tube. In step 9, the nuclei pellet was resuspended in 25ul cold PBS.
Immature ♀Gonad	70-80	The tissue was cut in small pieces in a petri dish with 800 ul HB buffer 1x (on ice).The tissue pieces with the liquid where transferred n a 1.5 ml eppendorf tube and mixed by pipetting (cut 1ml tip).Then the samples were immediately filtered through 70um cell strainer (if the previous treatment had homogenized the tissue enough), or they would pass through 1-2 passes with pestle B and then filtered.

<u>Transposase reaction and clean up</u>

12. In a new tube, the following transposase reaction mix was prepared:

Reagent	Volume per reaction (ul)
2x TD buffer	25
Illumina Transposase	2.5 (100 nM final)
Digitonin (1 %)	0.5 (1ul for gill)
Tween-20 (10 %)	0.5 (1ul for gill)
Nuclease free H2O	5 (4ul for gill)
Total	33.5

13. 33.5 ul transposase reaction mix were added to 16.5 ul of nuclei suspension and mixed by pipetting up and down 6 times.
14. The reaction was incubated at 37°C for 40' with 1000 RPM mixing. The next step was proceeded immediately
15. The transposation fragments were purified with MinElute Kit (QUIAGEN) , where the DNA was eluted in 23 ul elution buffer.
16. The concentration of the product was measured with Qubit BR (or Qubit HS in cases with lower concentrations).
17. The transposased DNA was stored at this point in -20°C until the library amplification.

Reagent	Volume per reaction (ul)
Illumina Nextera DNA UD Indexes (Set C)	5
NEBNext® Ultra™ II Q5® Master Mix	25
Sample (Transposed DNA)	20
Total	50

Initial PCR amplification

18. For every sample the following PCR reaction was prepared

19. PCR tubes were spun down
20. The following "Initial PCR amplification" program was run (the pre-heat option for lid set at 100˚C).

Temperature	Time	Cycles
72°C	5'	1
98°C	30''	1
98°C	10''	5
63°C	30''	
72°C	1'	
4°C	∞	

Determining the number of final PCR cycles with qPCR

21. 5 ul (10 %) of the Initially-amplified product were used to assemble 15 ul qPCR reactions to determine the appropriate number of additional cycles needed.
22. The following PCR reaction was made:

Reagent	Volume per reaction (ul)
NEBNext® Ultra™ II Q5® Master Mix	5
25X SYBRGreen (in H2O)	0.24
Illumina Nextera DNA UD Indexes (Set C)	1
Initial amplified DNA	5
Total	15

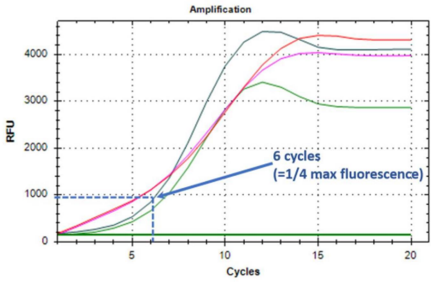
23. The nuclease free water, NEBNext® Ultra™ II Q5® Master Mix and 25X SYBRGreen were prepared in a qPCR mix for n+2 samples (n samples + 1 NTC + 1 additional). Then 9 ul from the qPCR mix were distributed to the qPCR tubes containing the initially amplified DNA and the same UD index as in the initial PCR.

24. The qPCR was run in a Magnetic Induction Cycler (Mic qPCR) with the following program:

Temperature	Time	Cycles
98°C	30''	1
98°C	10''	
63°C	30''	20
72°C	1'	
4°C	∞	

25. When qPCR was finished the number of addition cycles needed were determined with the following method:

a) 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.
b) If the ¼ value is between two cycle numbers, chose the lower one.
c) The number of cycles should equal ¼ of max fluorescence.



Final PCR amplification

26. The PCR tubes containing your Initially amplified product (45ul) were spun down
27. The "Final PCR amplification" program on your Initially amplified product (45ul) was run without adding anything:

Temperature	Time	Cycles
98°C	30''	1
98°C	10''	Additional cycles
63°C	30''	
72°C	1'	
4°C	∞	

28. After the Final PCR clean up with MinElute PCR purification column was performed to eliminate the majority of adaptor - dimers.

Library size selection

29. For removing the large and small fragments, NucleoMag kit for clean-up and size selection of NGS library prep reactions was used.
30. We followed the "Protocol for DNA double size selection". For removing the large fragments the ratio (ratio = volume beads/volume sample) was 0.53, and for removing small fragments the ratio was 0.9. The final library was eluted in 17 ul TET buffer.
31. The library concentration was measured with Qubit Broad Range Kit and the library was electrophoresed by 2100 Bioanalyzer system using the High sensitivity DNA Chip assay.
32. In case of an excess of long fragments in the samples (>1000 bp), an additional round of bead size selection was done.