



Tissue sampling for total RNA extraction, ATAC-seq and Chip-seq

Overview:

This protocol describes a method used to sample gilthead seabream tissues for isolation of small and large RNA with NucleoZOL (MACHEREY-NAGEL), for ATAC and ChIP libraries. Seven tissues were sampled from mature and immature *Sparus aurata* male and female individuals. More specifically the tissues were: liver, brain, muscle, gills, distal intestine, head kidney and gonad.

Consumables:

- Watchmaker forceps, Lab tweezers,
- Scalpel and blades, kitchen knives
- Labeled Eppendorf tubes and falcon tubes
- Aluminum foil
- Polystyrene box with dry ice
- Weighing balance
- Ethanol and distilled water to clean the tools
- Absorbent paper
- Pen and notepad

1. Fish have been starved for 24 hours prior to sampling.
2. The day of sampling fish were euthanized with an overdose of clove oil.
3. One person, would take one by one fish, and after taking required measurements of weight and length, the fish was photographed next to a ruler. The same person would start the dissection
4. Every organ that was removed was given to a different person (2-3 people in total), who would cut the tissue in multiple pieces and store it in labeled tubes (aliquots). The number and the size of aliquots were different, depending on the maturity/size of the fish and the specific organ.
5. The cutting was performed as quick as possible on benches covered with aluminum foil. After every tissue the foil was changed and the tools were cleaned with distilled water and ethanol.
6. The brain was sampled without the brain stem and the gills were sampled without the rakes and with the blood removed. The muscle tissue was sampled without any traces of skin. Finally the mucus was removed from the distal intestine and any traces of feces, since the fish were starved.
7. Since every organ was cut and stored in aliquots, it was given to an additional person who would snap freeze them on dry ice, and keep track of the sampling by noting which tissues have been stored.
8. By the end of the sampling additional AQUAFAANG metadata were recorded such as water salinity, fish? age, photo-period, etc.
9. The aliquots of the tissues were stored in -80°C for future use.