DevMap: Cell dissociation of gilthead seabream embryos and Cross-linking DNA for ChIP-Seq libraries

Overview: Both for ATAC and ChIP libraries, the protocols for the embryo extraction (DeVMap SOP-Embryo extraction) and the dissociation of embryonic cells (DevMap SOP-Cellular isolation of embryos) were altered to better serve their implementation in gilthead seabream's developmental stages. The size of the eggs, the dissimilarities of the location of the chorion and yolk sack between fresh and sea water species, as well as the observed lack of the embryo cellular coherence after testing the procedure of cell dissociation with watchmaker forceps, lead us to change these steps.

Below it is presented a method to obtain dissociated cells from gilthead seabream.

Consumables:

- Petri dish
- Pronase
- PBS
- 1% PFA solution
- 1M glycin solution
- 15ml falcon tubes
- Rotator system
- Dissection microscope
- 40μm filters (cell strainers)
- Centrifuge
- Trypan blue 0.4%
- 50ml conical tubes
    - Cell strainers are designed to universally fit most brands of 50mL conical tubes

1. Around 3000 eggs (~3 g), approximately two hours before the desired stage for blastula and early gastrula stage, and approximately one hour before the desired stage for 50% epiboly and onward stages, were transferred in a petri dish with clean salt water.

2. After water was removed, ~ 10 ml of pronase (3 mg/ml) was added in the petri dish with the eggs and incubated at 19 ^{o}C (with light steering[1]) until the desired stage was reached.

3. Pronase was removed and eggs were washed with PBS twice.

4. Dechorionation was performed in PBS using a pestle gently, and the solution was passed through a 40 µm cell strainer.

5. Cells in the flow-through were counted on a microscope in a hemocytometer after being dyed with Trypan blue (10 µl dye + 10 µl sample).

6. Cells were incubated with 875 µl of 1% PFA for 8-15 minutes, depending on the developmental stage (8 minutes for blastula and gastrula, 10 minutes for segmentation stages and 15 minutes after segmentation stages).

7. PFA was then inactivated with 125ul of 1M glycine.

8. The cells were pelleted with centrifugation (12 minutes at 300 x g, at 4 ^{o}C), re-suspended in 100µl PBS, and stored at -80^{o}C.

[1] In Lab oscillator/hula mixer/swinger