DevMap: Cell dissociation of gilthead seabream embryos and slow freezing for ATAC-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. The first section refers to earlier stages (blastula and early gastrula) and the second section refers to later stages (50% epiboly and later stages).

Consumables:

- 0.25% Trypsin EDTA
- Pronase
- PBS 1x
- 15ml falcon tubes
- Rotator system
- Dissection microscope
- FBS (Heat inactivated)
- DMEM
- DMSO
- 70 μm and 40μm filters (cell strainers)
- 2ml cryotubes
- MrFrosty cooling chamber
- Isopropyl alcohol (to fill the Mr Frosty cooling chambers, according to manufacturer's instructions)
- Centrifuge
- Trypan blue 0.4%
- 50ml conical tubes cryopreservation solution (45% DMEM, 55%FBS)

- Cell strainers are designed to universally fit most brands of 50mL conical tubes

1. Blastula and gastrula

1.1 Around 3000 eggs (~3 g), in case of blastula and gastrula stage, approximately two hours before the desired stage were transferred in a petri dish with clean salt water.

1.2 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.

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

1.4 For the early stages, the dechorionation was performed in the cryopreservation buffer by gentle use of a pestle, and the solution was passed through a 40 μm filter.

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

1.6 Solutions with early stages were filtered through 40 μm cell strainer

1.7 The volume of the flow through was split into ml cryotubes, and the volume was adjusted to 900 μl with cryopreservation solution.

1.8 DMSO was added, 100 μl to every 2 ml tube and put in MrFrosty.

2. 50% epiboly and later stages

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

2.2 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 until the desired stage was reached.

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

2.4 For later stages the dechorionation was performed in PBS with the use of pestle.

2.5 This time the solution was filtered through a 70 μm cell strainer.The filtrate was centrifuged (15', 300g, 4 ^{o}C), and the pellet was washed with PBS.

2.6 The centrifugation and the washing were repeaed one more time.

2.7 After the second wash the PBS was removed completely and the pellet was resuspended in Trypsin. The solution was transferred in 1.5ml falcon tube and Trypsin was added until the final volume of the solution was 3.5ml.

[1] In Lab oscillator/hula mixer/swinger

2.8 The embryos with Trypsin were incubated in a swinger (at 19 ^{o}C), for 20-60 min, depending on the developmental stage[2].

2.9 After the incubation Trypsin was deactivated with FBS (500µl FBS for 1ml Trypsin).

2.10 The solution was additionally filtered through 40 µm cell strainer.

2.11 The flow through was split in 1.5ml tubes and centrifuged (15', 300 g, 4 °C). The supernatant was carefully removed and the pellet was washed with 1ml PBS.

2.12 After the PBS wash the samples were centrifuged (15', 300g, 4 °C). The supernatant was carefully removed and in each tube the pellet was resuspended in, 900 µl cryopreservation solution.

2.13 Cells were counted after dying the with Trypan blue (as in previous stages). DMSO was added (100 µl in each tube) and the mix was transferred to 2 ml cryo tubes and put in MrFrosty.

[2] 50% epiboly/early somitogenesis: 20'-25', Mid somitogenesis:30', Late somitogenesis: 45', Pharyngula:50'-60'